NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF ACRYLONITRILE

(CAS NO. 107-13-1)

IN B6C3F$_1$ MICE

(GAVAGE STUDIES)

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

October 2001

NTP TR 506

NIH Publication No. 02-4440

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 Technical 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. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical 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 carcinogenic potential.

Details about ongoing and completed NTP studies are available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov. Abstracts of all NTP Technical Reports and full versions of the most recent reports and other publications are available from the NIEHS' Environmental Health Information Service (EHIS) http://ehis.niehs.nih.gov (800-315-3010 or 919-541-3841). In addition, printed copies of these reports are available from EHIS as supplies last. A listing of all the NTP Technical Reports printed since 1982 appears on the inside back cover.

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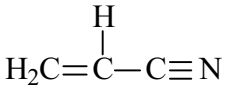
ABSTRACT



ACRYLONITRILE

CAS No. 107-13-1

Chemical Formula: C_3H_3N Molecular Weight: 53.06

Synonyms: Acrylonitrile monomer; cyanoethene; cyanoethylene; inhibited acrylonitrile; 2-propenenitrile; propenonitrile; vinyl cyanide
Trade names: Acritet, Acrylon, Carbacryl, ENT 54, Fumigrain, Miller's Fumigrain, Ventox

Acrylonitrile is used in the production of acrylic and modacrylic fibers, elastomers, acrylonitrile-butadiene-styrene and styrene-acrylonitrile resins, nitrile rubbers, gas barrier resins, and chemical intermediates such as adiponitrile and acrylamide. Acrylonitrile was nominated for study by the National Institute of Environmental Health Sciences because of its potential for human exposure, its classification as a probable human carcinogen, evidence of its carcinogenicity in rats, and the lack of carcinogenicity studies in a second animal species. Male and female B6C3F$_1$ mice received acrylonitrile (greater than 99% pure) in deionized water by gavage for 14 weeks or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium,* L5178Y mouse lymphoma cells, cultured Chinese hamster ovary cells, *Drosophila melanogaster,* and mouse peripheral blood erythrocytes.

14-Week Study

Groups of 10 male and 10 female mice were administered 0, 5, 10, 20, 40, or 60 mg acrylonitrile/kg body weight in deionized water by gavage, 5 days per week, for 14 weeks. All male and nine female mice in the 60 mg/kg groups and eight male and three female mice in the 40 mg/kg groups died on the first day of the study. The mean body weight gain of 20 mg/kg males was less than that of the vehicle control group. Clinical findings included lethargy and abnormal breathing in the 40 mg/kg groups. Leukocyte and lymphocyte counts were decreased in 20 mg/kg males and 40 mg/kg females, and a minimal hemolytic anemia was observed in 40 mg/kg females. Heart weights of 20 mg/kg males were significantly greater than those of the vehicle controls, and left cauda epididymis weights of 10 and 20 mg/kg males were significantly increased. The incidences of chronic active inflammation and hyperplasia in the forestomach of 40 mg/kg females were significantly increased.

2-Year Study

Groups of 50 male and 50 female mice were administered acrylonitrile in deionized water by gavage at doses of 0, 2.5, 10, or 20 mg/kg, 5 days per week, for 104 to 105 weeks. Urine from five male and five female mice from each group was collected at 2 weeks and at 3, 12, and 18 months and analyzed for thiocyanate and *N*-acetyl-S-(2-cyanoethyl)-L-cysteine concentrations as markers of exposure to acrylonitrile.

Survival, Body Weights, and Urinary Metabolite Analyses

Survival of 20 mg/kg mice was significantly less than that of the vehicle control groups. Mean body weights of 20 mg/kg males and females were generally less than those of the vehicle controls throughout most of the study. Dose-related increases in urinary thiocyanate and *N*-acetyl-S-(2-cyanoethyl)-L-cysteine concentrations occurred in all dosed groups at 2 weeks and at 3, 12, and 18 months.

Pathology Findings

The incidences of squamous cell papilloma, squamous cell carcinoma, and squamous cell papilloma or carcinoma (combined) of the forestomach occurred with positive trends in males and females, and were present in 50% or greater of mice administered 10 or 20 mg/kg. The incidences of mild focal or multifocal epithelial hyperplasia (combined) of the forestomach in 20 mg/kg males and females and of mild diffuse or focal hyperkeratosis (combined) in 20 mg/kg males were increased. The incidences of harderian gland adenoma and adenoma or carcinoma (combined) were significantly increased in all dosed groups of males and in 10 and 20 mg/kg females, and the incidence of harderian gland hyperplasia was significantly increased in 10 mg/kg males.

The incidence of benign or malignant granulosa cell tumor (combined) in the ovary of 10 mg/kg females was greater than that in the vehicle controls. The incidences of atrophy and cyst in the ovary of 10 and 20 mg/kg females were significantly increased. The incidence of alveolar/bronchiolar adenoma or carcinoma (combined) in 10 mg/kg females was significantly increased.

Genetic Toxicology

Acrylonitrile was mutagenic in *S. typhimurium* strains TA100 and TA1535 in the presence of S9 liver enzymes; it was not mutagenic without S9 activation in these two strains. No mutagenic activity was observed in strain TA97 or TA98 with or without S9. Acrylonitrile was mutagenic in mouse lymphoma L5178Y cells in the absence of S9; it was not tested with S9. In cultured Chinese hamster ovary cells, acrylonitrile induced sister chromatid exchanges with and without S9; chromosomal aberrations were significantly increased in the presence of S9 only. Tests for induction of sex-linked recessive lethal mutations in germ cells of male *D. melanogaster* were negative when acrylonitrile was administered in feed or by injection. A test for induction of reciprocal translocations in male *D. melanogaster* was negative. In contrast to the induction of chromosomal damage by acrylonitrile in mammalian cells *in vitro*, no increase in the frequency of micronucleated normochromatic erythrocytes was observed in peripheral blood samples from male or female mice administered acrylonitrile by gavage for 14 weeks. In summary, acrylonitrile induced genetic damage *in vitro* in bacterial and mammalian cells, but *in vivo* test results in *D. melanogaster* and in mice were negative.

Conclusions

Under the conditions of this 2-year gavage study, there was *clear evidence of carcinogenic activity** of acrylonitrile in male and female $B6C3F_1$ mice based on increased incidences of forestomach and harderian gland neoplasms. Neoplasms of the ovary and lung in female mice may have been related to administration of acrylonitrile.

Nonneoplastic lesions of the forestomach and harderian gland in males and of the forestomach and ovary in females were associated with administration of acrylonitrile by gavage for 2 years.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 8. A summary of the Technical Reports Review Subcommittee comments and public discussion on this Technical Report appears on page 10.

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

	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Doses in deionized water by gavage	Vehicle control, 2.5, 10, or 20 mg/kg	Vehicle control, 2.5, 10, or 20 mg/kg
Survival rates	38/50, 42/50, 39/50, 14/50	39/50, 32/50, 39/50, 23/50
Body weights	20 mg/kg group generally less than vehicle control group	20 mg/kg group generally less than vehicle control group
Nonneoplastic effects	Forestomach: epithelial hyperplasia, focal (2/50, 4/50, 8/50, 9/50); hyperkeratosis, diffuse or focal (2/50, 3/50, 7/50, 12/50) Harderian Gland: hyperplasia (1/50, 4/50, 7/50, 4/50)	Forestomach: epithelial hyperplasia, focal or multifocal (2/50, 2/50, 5/50, 7/50) Ovary: atrophy (6/50, 8/50, 45/50, 40/50); cyst (12/50, 20/50, 27/50, 19/50)
Neoplastic effects	Forestomach: squamous cell papilloma (3/50, 4/50, 19/50, 25/50); squamous cell carcinoma (0/50, 0/50, 8/50, 9/50); squamous cell papilloma or carcinoma (3/50, 4/50, 26/50, 32/50) Harderian Gland: adenoma (5/50, 16/50, 24/50, 27/50); adenoma or carcinoma (6/50, 16/50, 27/50, 30/50)	Forestomach: squamous cell papilloma (3/50, 6/50, 24/50, 19/50); squamous cell carcinoma (0/50, 1/50, 1/50, 11/50); squamous cell papilloma or carcinoma (3/50, 7/50, 25/50, 29/50) Harderian Gland: adenoma (10/50, 10/50, 25/50, 23/50); adenoma or carcinoma (11/50, 10/50, 26/50, 25/50)
Equivocal findings	None	Ovary: benign or malignant granulosa cell tumor (0/50, 0/50, 4/50, 1/50) Lung: alveolar/bronchiolar adenoma or carcinoma (6/50, 6/50, 14/50, 9/50)
Level of evidence of carcinogenic activity	Clear evidence	Clear evidence

Genetic toxicology

Salmonella typhimurium gene mutations:	Positive in strains TA100 and TA1535 with S9; negative in TA97 and TA98, with and without S9
Mouse lymphoma gene mutations:	Positive without S9
Sister chromatid exchanges	
Chinese hamster ovary cells *in vitro:*	Positive with and without S9
Chromosomal aberrations	
Chinese hamster ovary cells *in vitro:*	Positive with S9, negative without S9
Sex-linked recessive lethal mutations	
Drosophila melanogaster:	Negative
Reciprocal translocations	
Drosophila melanogaster:	Negative
Micronucleated erythrocytes	
Mouse peripheral blood *in vivo:*	Negative

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

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

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

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

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on acrylonitrile on 3 May 2001 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

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

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 3 May 2001, the draft Technical Report on the toxicology and carcinogenesis studies of acrylonitrile received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. B.I. Ghanayem, NIEHS, introduced the toxicology and carcinogenesis studies of acrylonitrile by discussing the uses of the chemical and the rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in mice. Dr. Ghanayem noted that acrylonitrile is a recognized carcinogen in several strains of rats. The proposed conclusion for the 2-year study was *clear evidence of carcinogenic activity* in male and female mice.

Dr. Drinkwater, the first principal reviewer, inquired about details of urinary metabolite collection and the apparent increase in excretion of two metabolites with age. Dr. Ghanayem replied that while there was increased variation with age, examination of 15 metabolites and creatinine did not reveal a consistent pattern of change.

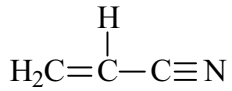
Dr. Chatman, the second principal reviewer, asked about the classification of the ovary and lung neoplasms as "uncertain" findings in the summary table. Dr. J.R. Bucher, NIEHS, said that the subheading in the table would be changed to "equivocal." Dr. Chatman questioned the appropriateness of grouping various types of ovarian cysts for analysis. Dr. A. Nyska, NIEHS, explained that the cell of origin of cysts, particularly in older animals, cannot be determined, and the program policy is to combine them under one category.

Dr. Dragan, the third principal reviewer, suggested that pharmacokinetic data could be helpful in establishing doses for long-term studies. Dr. Ghanayem replied that for this particular chemical, the acute toxicity due to formation of cyanide as a metabolite was the determining factor in dose selection.

Dr. Thrall asked if a decrease in hematocrit in female mice could be attributed to Heinz body formation and also commented on the lack of hematology measures in the long-term studies. Dr. G.S. Travlos, NIEHS, said that Heinz bodies were indeed looked for but were not detected. He explained that hematology measurements were limited in 2-year studies to avoid bleeding in core study animals and also because disease processes in older animals made such measures more variable and less amenable to interpretation.

Dr. Dragan moved, and Dr. Drinkwater seconded, that the conclusion be modified to indicate that the neoplasms of the ovary and lung in female mice were not dose-dependent. After discussion among the panel, the motion was withdrawn. Dr. Drinkwater then moved that the conclusion be accepted as written for male and female mice, *clear evidence of carcinogenic activity*. Dr. Dragan seconded the motion, which was approved with seven votes and one abstention (Dr. Klaunig).

INTRODUCTION



ACRYLONITRILE

CAS No. 107-13-1

Chemical Formula: C_3H_3N Molecular Weight: 53.06

Synonyms: Acrylonitrile monomer; cyanoethene; cyanoethylene; inhibited acrylonitrile; 2-propenenitrile; propenonitrile; vinyl cyanide
Trade names: Acritet, Acrylon, Carbacryl, ENT 54, Fumigrain, Miller's Fumigrain, Ventox

Chemical and Physical Properties

Acrylonitrile is a colorless, volatile, flammable, and explosive liquid with a weak pungent odor. On standing, it may develop a yellowish color after exposure to light. It is a polar molecule because of the presence of the CN group and is miscible in water and most organic solvents. Acrylonitrile has a boiling point of 77.3° C at 760 mm Hg, a melting point of -83.55° C, and a density of 0.8060 at 20° C (*Merck Index,* 1996; Lide, 2000). It has a vapor pressure of 13.3 Kpa at 23° C and an open cup flash point of 0° C. Acrylonitrile may polymerize spontaneously, particularly in the absence of oxygen or when exposed to light, and it polymerizes violently in the presence of concentrated alkali (*Merck Index,* 1996).

Production, Use, and Human Exposure

Acrylonitrile is prepared by dehydration of ethylene cyanohydrin or acrylamide by phosphorus pentoxide (Brazdil, 1999). Most current commercial production of acrylonitrile occurs by the ammoxidation of propylene, which involves the selective oxidation of propylene and ammonia (*Merck Index,* 1996).

Worldwide production of acrylonitrile in 1988 was approximately 3.2 million tons (IARC, 1999). The major uses of acrylonitrile involve the production of acrylic and modacrylic fibers, elastomers, acrylonitrile-butadiene-styrene and styrene-acrylonitrile resins, nitrile rubbers, gas barrier resins, and chemical intermediates such as adiponitrile and acrylamide (Brazdil, 1999; IARC, 1999).

Human exposure to acrylonitrile in the environment is minimal because when acrylonitrile is released in air or water, it is broken down. In the atmosphere, the calculated half-life of acrylonitrile based on hydroxy radical reaction rate constants varied from 4 to 189 hours (Callahan *et al.,* 1979; USEPA, 1980; Edney *et al.,* 1982; Howard, 1989; Grosjean, 1990). The half-life in water also varied from 30 to 552 hours based on aqueous biodegradation (Going *et al.,* 1979; Howard *et al.,* 1991). Based on volatilization, the half-life of acrylonitrile is 1 to 6 days (Howard *et al.,* 1991). Human exposure to acrylonitrile occurs primar-ily in the workplace or in areas adjacent to acrylonitrile plants (IARC, 1999). From 1981 to 1983, an estimated 80,000 workers were potentially exposed to acrylonitrile in the United States (NIOSH, 1990). In the 15 countries of the European Union, it is estimated that approximately 35,000 workers were potentially exposed to acrylonitrile (IARC, 1999). The American Conference of Governmental Industrial Hygienists (2000) recommends a threshold limit value time-weighted average (TWA) of 4.3 mg/m^3 for acrylonitrile. Similarly, the Occupational Safety and Health Administration's permissible limit is 4.3 mg/m^3 for an 8-hour TWA with a 15-minute ceiling that is not to exceed 21.5 mg/m^3 during the work day (NIOSH, 1997).

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

Regardless of the route of administration, acrylonitrile is rapidly absorbed and distributed to all major tissues in mammals. Acrylonitrile is eliminated primarily in the urine after exposure of rats and mice to acrylonitrile orally, intravenously, intraperitoneally, dermally, or by inhalation (Young *et al.,* 1977; Sapota*,* 1982; Kedderis *et al.,* 1993a; Burka *et al.,* 1994). Urinary elimination of acrylonitrile metabolites accounted for 77% to 104% of the dose 72 hours after rats and mice were administered 0.09 to 29 mg/kg by gavage (Kedderis *et al.,* 1993a). Administration of 46 mg/kg resulted in the elimination of 67% of the dose in the urine after 24 hours (Burka *et al.,* 1994). Elimination of acrylonitrile-derived radioactivity also occurred in the expired air as carbon dioxide and accounted for 11% of a 46 mg/kg gavage dose after 24 hours (Burka *et al.,* 1994) and 4% to 5% of a 10 mg/kg gavage dose after 72 hours (Young *et al.,* 1977). Fecal elimination accounted for 11% within 24 hours after a gavage dose of 46 mg/kg (Burka *et al.,* 1994) and 3% to 5 % of the 10 mg/kg gavage dose after 72 hours (Young *et al.,* 1977; Kedderis *et al.,* 1993a).

Tissue distribution of acrylonitrile and/or its metabolites after administration by various routes demonstrated that there is no potential for acrylonitrile bioaccumulation in any animal tissue (Young *et al.,* 1977; Sapota*,* 1982; Burka *et al.,* 1994). Acrylonitrile and/or its metabolites were distributed to all major tissues examined. Relatively higher concentrations of acrylonitrile-derived radioactivity were detected in the lung, kidney, stomach, skin, and packed red blood cells than in other tissues of rats exposed to acrylonitrile by inhalation or gavage (Young *et al.,* 1977; Ahmed *et al.,* 1982). After a 46 mg/kg gavage dose to rats, high levels of acrylonitrile and/or its metabolites were found in the blood, intestines, kidney, liver, lung, stomach, and urinary bladder (Silver *et al.,* 1987; Burka *et al.,* 1994). Comparison of tissue distribution after gavage versus intravenous administration showed that high levels of acrylonitrile and/or its metabolites in the stomach were present after administration by either route (Young *et al.,* 1977; Burka *et al.,* 1994). Detection of high levels of ^{14}C-acrylonitrile-derived radioactivity in the forestomach after intravenous and gavage dosing suggests that retention of radioactivity in the stomach is not caused by poor absorption but by enterogastric circulation. Retention of acrylonitrile and/or its metabolites in the red blood cells of rats dosed with acrylonitrile by gavage lasted as long as 10 days, despite the fact that plasma levels declined significantly at a much earlier time (Ahmed *et al.,* 1982). This retention was attributed to covalent binding of acrylonitrile and/or its metabolites to hemoglobin. The hemoglobin adduct, N-(2-cyanoethyl)valine of acrylonitrile was detected in the blood of rats exposed directly to acrylonitrile (Gargas *et al.,* 1995) and in humans exposed to acrylonitrile by cigarette smoking (Bergmark, 1997; Fennel *et al.,* 2000)

Acrylonitrile is metabolized via two major pathways (Figure 1). The first pathway involves the direct conjugation of acrylonitrile with reduced glutathione by a simple Michael addition. It is not clear if this conjugation is catalyzed by glutathione transferases; the high reactivity of acrylonitrile suggests that it may occur nonenzymatically (Burka *et al.,* 1994). This conjugate was detected in the bile of rats treated with acrylonitrile (Ghanayem and Ahmed*,* 1982). Subsequent degradation of this metabolite leads to the formation and urinary excretion of *N*-acetyl-S-(2-cyanoethyl)cysteine (Fennell *et al.,* 1991; Burka *et al.,* 1994; Sumner *et al.,* 1999).

The second pathway involves the oxidative metabolism of acrylonitrile leading to the formation of the epoxide intermediate, 2-cyanoethylene oxide (glycidonitrile) (Figure 1). Toxicokinetic studies demonstrated that acrylonitrile epoxidation to 2-cyanoethylene oxide is saturable and may follow simple Michaelis-Menten saturation kinetics (Roberts *et al.,* 1989, 1991; Kedderis *et al.,* 1993b; Gargas *et al.,* 1995). Results of earlier studies suggested that, while cytochrome P4502E1 (CYP2E1) is a major enzyme responsible for acrylonitrile epoxidation, other P450s can also metabolize acrylonitrile (Guengerich *et al.,* 1991; Kedderis *et al.,* 1993c). More recently, studies using CYP2E1 knockout mice demonstrated that CYP2E1 is the only P450 responsible for acrylonitrile epoxidation (Sumner *et al.,* 1999; Ghanayem *et al.,* 2000). In an *in vitro* study, acrylonitrile epoxidation may have been catalyzed by soybean and human lung lipooxygenase (Roy and Kulkarni, 1999). However, no *in vivo* evidence is available to support this finding.

Subsequent metabolism of 2-cyanoethylene oxide occurs mainly via conjugation with glutathione, which may occur at the number 2 or number 3 carbon atoms (Figure 1). Conjugation at the number 3 position yields cyanohydrin, which may undergo further metabolism to produce *N*-acetyl-S-(carboxymethyl)cysteine, *N*-acetyl-S-(2-hydroxyethyl)cysteine, and thionyldiacetic acid. Reduced glutathione conjugation with 2-cyanoethylene

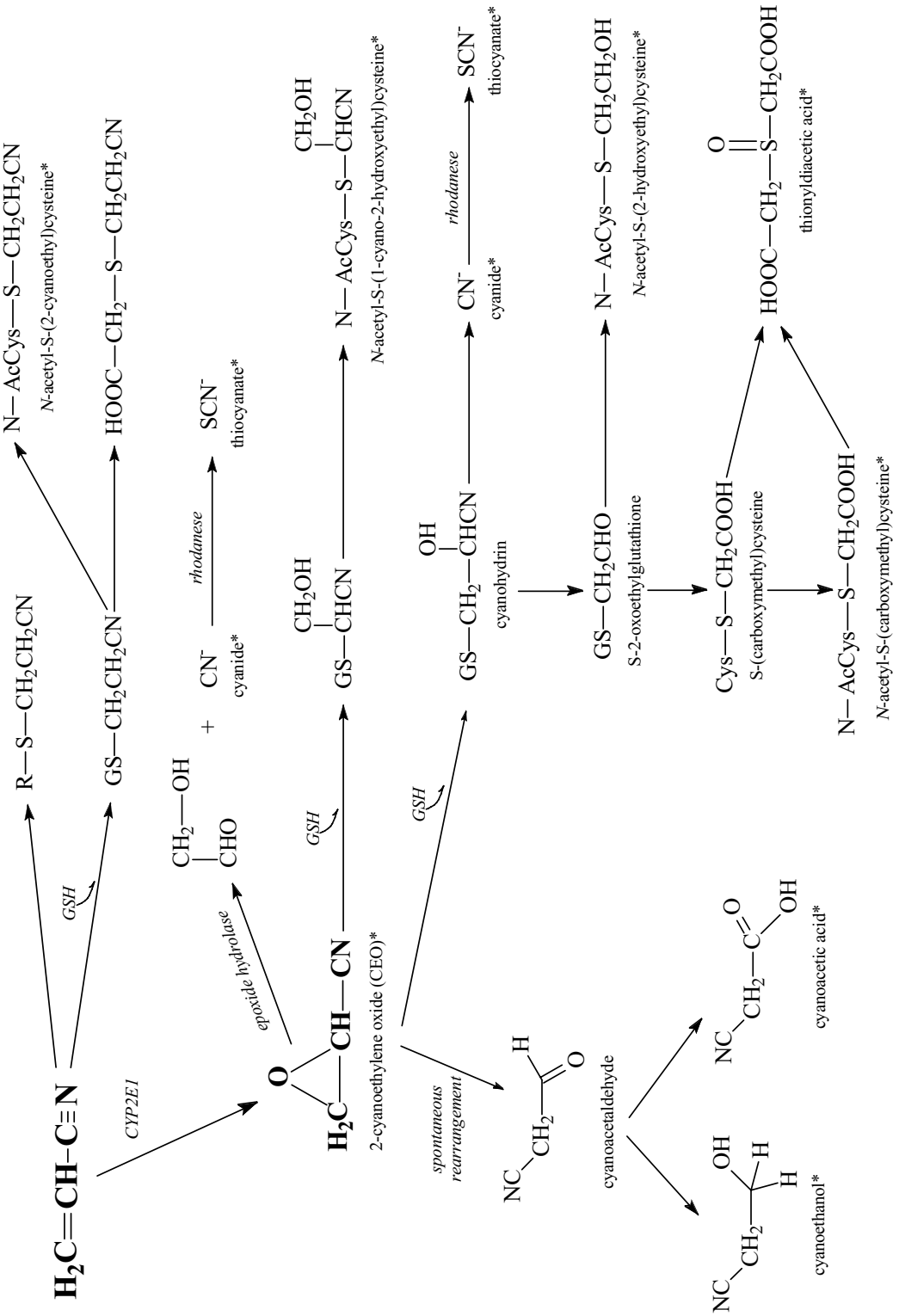


FIGURE 1
Summary of Acrylonitrile Metabolism
(*=confirmed metabolite)

oxide at the number 2 carbon atom leads to the formation and excretion of *N*-acetyl-S-(1-cyano-2-hydroxyethyl)-cysteine in the urine. 2-Cyanoethylene oxide may also be the subject of a rearrangement leading to the formation of cyanoacetaldehyde, which eventually leads to the formation and urinary excretion of cyanoethanol and cyanoacetic acid.

Acrylonitrile metabolism also results in the liberation of cyanide, which is converted to thiocyanate in a reaction catalyzed by the rhodanese enzyme (Figure 1). Liberation of cyanide from acrylonitrile is thought to proceed via 2-cyanoethylene oxide formation. *In vitro* studies using liver microsomes demonstrated that acrylonitrile metabolism to cyanide is catalyzed by the cytochrome P450 enzymes (Abreu and Ahmed, 1980; Guengerich *et al.,* 1981). It is likely that 2-cyanoethylene oxide hydrolysis via the epoxide hydratase contributes to cyanide release from acrylonitrile. There were no significant differences in cyanide levels in the blood and tissues of CYP2E1 knockout mice that received acrylonitrile versus the vehicle-treated knockout mice (Ghanayem *et al.,* 2001). In contrast, blood cyanide levels increased in a dose-dependent manner in wild-type mice treated with acrylonitrile. These data provided direct evidence that cyanide formation from acrylonitrile is preceded by its metabolism to 2-cyanoethylene oxide exclusively via CYP2E1.

Significant species-dependent variations in the metabolism and disposition of acrylonitrile have been reported. The ratio of acrylonitrile epoxidation to direct glutathione conjugation is greater in mice than in rats (Kedderis *et al.,* 1995). Further, hepatic microsomes of mice metabolized acrylonitrile to 2-cyanoethylene oxide at a greater rate than did those of rats or humans, which epoxidized acrylonitrile at approximately the same rate (Kedderis and Batra, 1993; Kedderis *et al.,* 1993c). Mice also excreted a significantly greater percentage of the dose in the form of metabolites originating from 2-cyanoethylene oxide (Fennell *et al.,* 1991) and excreted more urinary thiocyanates (Gut *et al.,* 1975) than rats.

Acrylonitrile metabolism and disposition are also inflenced by the route of administration. The percentage of dose excreted by rats in the urine as thiocyanate was greater after exposure by inhalation or by gavage administration than after intravenous or intraperitoneal injetion (Gut *et al.,* 1975, 1981; Tardif *et al.,* 1987). The port of entry of administered acrylonitrile apparently plays a role in its metabolism. While *N*-acetyl-S-(2-cyanoethyl)cysteine was the major urinary metabolite after intraperitoneal injection, it represented a minor metabolite after inhalation exposure (Tardif *et al.,* 1987). The effect of the route of administration on the metabolism of acrylonitrile may be attributed to the tissue glutathione content subjected to the first-pass effect (i.e., lung after inhalation and liver after gavage administration or intraperitoneal injection) (Léonard *et al.,* 1999).

Humans

Volunteers exposed to 20 mg/m^3 acrylonitrile in the atmosphere for up to 4 hours showed a respiratory retention of approximately 46% (Rogaczewska and Piotrowski, 1968). In another study, exposure to acrylonitrile in air for 8 hours resulted in the retention of 52% of the dose, and approximately 22% of the retained portion of the dose was eliminated in the urine as *N*-acetyl-S-(2-cyanoethyl)-L-cysteine (Jakubowski *et al.,* 1987). Controlled dermal studies using neat acrylonitrile application to the forearm of human volunteers suggested that the dermal absorption rate of acrylonitrile was approximately 0.6 mg/cm^3 per hour (Rogaczewska and Piotrowski, 1968). Factory workers exposed to acrylonitrile vapors excreted unchanged acrylonitrile in their urine (Houthuijs *et al.,* 1982). However, no other animal or human studies confirmed the excretion of unchanged acrylonitrile in the urine after exposure.

TOXICITY

Experimental Animals

Acrylonitrile is a potent toxicant. The acute toxicity of acrylonitrile is generally attributed to cyanide, P450-formed oxidative metabolites, and glutathione depletion.

The acute toxicity of acrylonitrile varies significantly as a function of the route of administration and animal species with dogs being most sensitive followed by mice, rabbits, cats, rats, and guinea pigs (USEPA, 1983; IARC, 1999). The reported oral LD_{50} of acrylonitrile after gavage administration varies from 78 to 150 mg/kg in rats (Wilson *et al.,* 1948; Benesh and Cherna, 1959; Knobloch *et al.,* 1971) and 20 to 27 mg/kg in mice (Benesh and Cherna, 1959). The 4-hour LC_{50} of acrylonitrile in mice is 300 to 900 mg/m^3 (Knobloch *et al.,* 1971, 1972). Recent studies suggest that the LD_{50} of acrylonitrile is higher than previously reported; an estimate using the up-and-down method suggest that the oral LD_{50} in $B6C3F_1$ mice is much higher than previously reported (Ghanayem, unpublished data).

Depending on the dose and species, the symptoms of acrylonitrile toxicity are qualitatively similar among laboratory animals. The symptoms of acute acrylonitrile toxicity include early acetylcholine-like toxicity such as miosis, chromodacryorrhea, lacrimation, salivation, nasal discharge, vasodilatation (evident by the reddening of the ears, face, and extremities), vomiting, and diarrhea; this early stage of toxicity is usually followed by delayed cyanide-like effects including depression, rapid and shallow breathing, apnea, and convulsions followed by death when acrylonitrile is administered at high doses (Paulet and Desnos, 1961; Graham, 1965; Paulet *et al.,* 1966; Ghanayem *et al.,* 1991).

Various treatments were assessed as antidotes for the acute toxicity of acrylonitrile. Atropine sulfate was effective in alleviating the early cholinomimetic toxicity of acrylonitrile (Ghanayem *et al.,* 1991). Conventional cyanide antidotes and sulfhydryl-containing compounds were also effective in alleviating the early symptoms of acrylonitrile toxicity (Hashimito and Kani, 1965; Ghanayem and Ahmed*,* 1986).

Acrylonitrile was found to covalently bind with hemoglobin and erythrocyte membranes (Farooqui and Ahmed, 1983a). Acrylonitrile caused acute hemolytic anemia, as evidenced by a decreased number of circulating erythrocytes and a decrease in hematocrit and hemoglobin concentration (Farooqui and Ahmed, 1983b).

Mild irritation and conjunctivitis were observed in the eye of rabbits after a single-drop application (McCormie *et al.,* 1949). Topical application of neat acrylonitrile resulted in localized vasodilatation, edema, and hyperventilation (McCormie *et al.,* 1949; Zeller *et al.,* 1969).

Gavage and subcutaneous administration of acrylonitrile resulted in dose-dependent mucosal hemorrhage and erosions of the glandular stomach in association with glutathione depletion; these effects were ameliorated by atropine and sulfhydryl compounds (Ghanayem *et al.,* 1985; Ghanayem and Ahmed, 1986). Gavage administration of 25 mg/kg acrylonitrile to rats once per day for 10 days resulted in thickening of the forestomach (Murray *et al.,* 1978). Administration of acrylonitrile to male F344 rats by gavage at 0.22, or 0.43 mmol/kg for 6 weeks resulted in a selective dose-dependent enhancement of mucosal cell proliferation of the forestomach (Ghanayem *et al.,* 1997). This effect was also associated with a dose-dependent increase in the thickness of the forestomach; acrylonitrile had no effect on cell proliferation in the liver or glandular stomach.

Administration of a single intravenous 100 mg/kg acrylonitrile injection to dogs caused pulmonary edema (Graham, 1965). Additionally, Ahmed *et al.* (1992) reported that a single gavage administration of 46.5 mg/kg to Sprague-Dawley rats resulted in lung clara cell hyperplasia.

Acrylonitrile administered at lethal doses by gavage or intravenous injection to rats resulted in acute adrenal apoplexy (Szabo *et al.,* 1983). Acrylonitrile also caused duodenal ulcers in these rats. Female F344 rats exposed to 100 or 500 ppm acrylonitrile in drinking water for 12 to 18 months had significant neurological signs of toxicity including decreased activity, paralysis, head tilt, circling, and seizures (Bigner *et al.,* 1986). In a later study, rats exposed to 25 ppm acrylonitrile for 24 weeks by inhalation had reversible reductions in motor and sensory conduction (Gagnaire *et al.,* 1998).

A study using Sprague-Dawley rats exposed to 0, 5, 10, 100, or 200 ppm acrylonitrile in drinking water for 14, 28, or 90 days revealed that acrylonitrile increased oxidative DNA damage in the brain, evidenced by the presence of 8-hydroxy-2′-deoxyguanosine, products of lipid peroxidation, and reactive oxygen species (Jiang *et al.,* 1998). No changes in these parameters were reported in the liver of these animals. Additionally, *in vitro* incubation of high concentrations of acrylonitrile with a rat glial cell line or hepatocytes showed that 8-hydroxy-2′-deoxyguanosine and hydroxy radical formation increased in glial cells but not in hepatocytes (Kamendulis *et al.,* 1999a). In recent studies, acrylonitrile caused dose-dependent inhibition of gap junction intercellular communication in a rat astrocytes cell line (Kamendulis *et al.,* 1999b), a property that is considered more characteristic of nongenotoxic substances.

Humans

In the workplace, the most likely routes of human exposure to acrylonitrile are dermal or inhalation. The acute toxicity of inhaled acrylonitrile is manifested as nausea, vomiting, nasal and eye irritation, headache, fatigue, irritability, mild jaundice, mild anemia, and tremors (Wilson, 1944; Zeller *et al.,* 1969; Muto *et al.,* 1992). Dermal exposure to acrylonitrile caused skin irritation, painful itching, blistering, and allergic dermatitis (Zeller *et al.,* 1969; Balda 1975; Bakker *et al.,* 1991). One worker exposed to acrylonitrile dermally and by inhalation suffered from dizziness, nausea, vomiting, increased serum transaminases, and myoglobinurea (Vogel and Kirkendall, 1984).

Reproductive Toxicity and Teratogenicity

Experimental Animals

Acrylonitrile (10 mg/kg) administered to CD1 mice by gavage for 60 days caused degenerative changes of the seminiferous tubules associated with a significant decline in sperm counts (Tandon *et al.,* 1988). Ahmed *et al.* (1992) reported that acrylonitrile administered by gavage to Sprague-Dawley rats may interfere with testicular DNA synthesis and repair. Female Sprague-Dawley rats dosed with 10, 25, or 65 mg/kg acrylonitrile per day by gavage or exposed to 40 or 80 ppm acrylonitrile for 6 hours per day by inhalation during days 6 to 15 of pregnancy showed maternal and fetal toxicity (Murray *et al.,* 1978). Significant increases in incidences of short tails or missing vertebrae were observed in 65 mg/kg fetuses, and lower incidences were observed in 80 ppm fetuses. The no-observed-adverse-effect levels on the embryo were 10 mg/kg (gavage) and 40 ppm (inhalation). Intraperitoneal injection of 80 to 120 mg/kg acrylonitrile to hamsters on day 8 of gestation resulted in exencephaly and rib malformations in the offspring (Willhite *et al.,* 1981); administration of sodium thiosulfate protected against teratogenicity induced by 80 mg/kg acrylonitrile, but not 120 mg/kg.

Female offspring of Sprague-Dawley rats exposed to 500 ppm acrylonitrile in drinking water had progressive muscle weakness in the hind limbs 16 to 19 weeks after the second litter was weaned (Svirbely and Floyd, 1961). Sprague-Dawley rats exposed to 12 to 100 ppm acrylonitrile by inhalation for 6 hours per day during gestation days 6 to 20 showed no significant teratogenicity (Saillenfait *et al.,* 1993a). *In vitro,* exposure of whole rat embryos to acrylonitrile resulted in glutathione depletion and aggravated acrylonitrile toxicity on the embryos (Saillenfait *et al.,* 1993b).

In a recent study with incubated whole rat embryos, acrylonitrile caused concentration-dependent decreases in growth and differentiation and increases in morphological abnormalities (Saillenfait and Sabate, 2000). The presence of liver microsomes and NADPH in the embryo cultures enhanced the embryotoxicity of acrylonitrile. *In vivo,* a single gavage dose of 100 mg/kg acrylonitrile to pregnant Sprague-Dawley rats on gestation day 10 resulted in embryo defects similar to those caused by sodium cyanide, including allantois, trunk, and/or misdirected caudal extremity.

In a three-generation reproductive toxicity study, Sprague-Dawley rats and their offspring (15 days post-weaning) were exposed to 100 or 500 ppm acrylonitrile in drinking water and were mated after 100 days (Beliles *et al.,* 1980); progressive muscle weakness in the hind limbs was observed in the offspring at 16 to 19 weeks.

Humans

No information on the reproductive toxicity or teratogenicity of acrylonitrile in humans was found in the literature.

Carcinogenicity

Experimental Animals

Oral gavage, drinking water, and inhalation studies in rats have been reported; most demonstrate that acrylonitrile causes cancer in rats. No studies assessing the carcinogenicity of acrylonitrile in other species were found in the literature.

Long-term exposure of female F344 rats to 100 or 500 mg acrylonitrile/L drinking water resulted in a significant increase in primary brain tumors (Bigner *et al.,* 1986). Tumors were also observed in the Zymbal's gland, forestomach, and skin of treated rats.

In another study, male Sprague-Dawley rats were exposed to 0, 20, 100, or 500 ppm acrylonitrile in the drinking water for 2 years (Gallagher *et al.,* 1988). Incidences of Zymbal's gland tumors were significantly increased in the 500 ppm group; forestomach tumors also occurred in the 500 ppm group.

In a study conducted by BioDynamics, Inc. (1980a), male and female Fischer rats were exposed to 0, 1, 3, 10, or 100 ppm acrylonitrile in the drinking water for 19 or 22 months; significant increases in the incidences of astrocytomas of the brain and tumors of the spinal cord (females only), Zymbal's gland, and forestomach occurred in 100 ppm males and females. In a second study conducted by BioDynamics (1980b), Spartan rats were exposed to 0, 1, 3, 10, 30, or 100 ppm acrylonitrile in the drinking water; the incidences of astrocytomas in the brain and tumors of the Zymbal's gland and forestomach in males and females were significantly increased.

Sprague-Dawley rats were administered 0 or 5 mg/kg acrylonitrile by gavage in olive oil once per day, 3 days per week, for 52 weeks and sacrificed at 131 weeks of age (Maltoni *et al.,* 1977). Slight increases in the incidences of tumors occurred in the mammary gland and forestomach. In a later report, Maltoni *et al.* (1988) reported that acrylonitrile was not carcinogenic when administered to Sprague-Dawley rats by gavage at 5 mg/kg in olive oil for 52 weeks (animals were examined at 131 weeks).

Carcinogenicity was assessed in male and female Spartan rats administered 0, 0.1 or 10 mg/kg acrylonitrile by gavage once per day for 2 years (BioDynamics, 1980c). There were significant increases in the incidences of tumors in the brain and Zymbal's gland of 10 mg/kg males and females. The incidences of forestomach and intestinal tumors were significantly increased in 10 mg/kg males, and the incidence of mammary gland tumors was significantly increased in 10 mg/kg females.

Significant increases in the incidences of mammary gland tumors occurred in female Sprague-Dawley rats exposed to 5, 10, 20, or 40 ppm acrylonitrile by whole-body inhalation for 4 hours per day, 5 days per week, for 52 weeks (Maltoni *et al.,* 1977). The incidences of forestomach and skin tumors were also increased, and Zymbal's gland tumors were present. Additionally, the incidences of benign and malignant tumors (combined) in exposed rats were significantly greater than those in the controls.

In a subsequent study, groups of male and female Sprague-Dawley rats were exposed to 0, 5, 10, 20, or 40 ppm acrylonitrile by inhalation 4 hours per day, 5 days per week, for 52 weeks. Increased incidences of Zymbal's gland and forestomach tumors and brain gliomas were reported (Maltoni *et al.,* 1988). In the same inhalation study, male and female Sprague-Dawley rat breeders were exposed to 60 ppm acrylonitrile for 104 weeks, and their 12-day-old pups were exposed to 60 ppm for 15 or 104 weeks. All rats were allowed to live out their life span and then examined. The incidences of mammary gland tumors as well as extrahepatic angiosarcoma were increased in female offspring exposed for 104 weeks, and Zymbal's gland and liver tumors were increased in exposed male offspring. The incidence of brain glial cell tumors also increased in males and females after exposure to acrylonitrile for 104 weeks.

Humans

Early epidemiology studies suggested an association between acrylonitrile exposure and human carcinogenicity. Occupational exposure to acrylonitrile resulted in increased incidences of lung cancers (Thiess *et al.,* 1980), colon cancers (Mastrangelo *et al.,* 1993), and all tumors (Zhou and Wang, 1991). Generally, these early epidemiology studies were inconclusive because they suffered from one or more of the following drawbacks: small cohort of workers, inadequate exposure assessment, potential confounding factors such as smoking, diet, exposure to more than a single chemical, and limited follow-up.

Epidemiology studies in exposed workers addressed many of these drawbacks and suggested that there is no significant association between exposure to acrylonitrile and carcinogenicity (Benn and Osborne, 1998; Swaen *et al.,* 1998; Wood *et al.,* 1998). In another study, mortality from lung cancer was slightly elevated in the group of workers exposed to the highest cumulative amount of acrylonitrile (Blair *et al.,* 1998). However, further analysis of the exposure-response relationships failed to confirm an association between exposure to acrylonitrile and lung cancer in humans.

Genetic Toxicity

The mutagenicity of acrylonitrile has been studied extensively. Acrylonitrile was investigated by a number of laboratories in several standard mutagenicity assays as part of an International Program on Chemical Safety (IPCS) collaborative study (Ashby *et al.,* 1985). Additional comprehensive reviews of the mutagenicity of acrylonitrile were provided by the International Agency for Research on Cancer (1987, 1999) and by Léonard *et al.* (1999). Based on the data summarized in these reviews, acrylonitrile was positive in bacterial gene mutation assays, yeast assays for chromosomal segregation effects, *Drosophila* and cultured mammalian cell gene mutation assays, and cultured mammalian cell assays for clastogenicity. Metabolism to the reactive metabolite, 2-cyanoethylene oxide, seems to be critical for acrylonitrile genotoxicity; therefore, experimental conditions that optimize the conversion or supply exogenous S9 liver enzymes are required to detect the genotoxic effects of acrylonitrile (Whysner *et al.,* 1998). Although positive results were obtained in a broad array of acrylonitrile *in vitro* assays, *in vivo* clastogenicity has not been demonstrated. This may indicate that the mutagenic metabolite is produced in small amounts, is very short-lived, or does not reach the target tissues.

The results of the IPCS collaborative study showed that acrylonitrile was mutagenic in bacterial assays, but responses among protocols were inconsistent (Venitt, 1985). In a preincubation study with S9, acrylonitrile was clearly positive in *Salmonella typhimurium* strains TA100 and TA1535 (Zeiger and Haworth, 1985). The results of previously published studies were consistent with the results of the IPCS collaborative study and indicated that acrylonitrile was a bacterial mutagen with activity primarily in base-pair substitution strains of *S. typhimurium*. Most studies indicated a requirement for S9 enzymes (de Meester *et al.,* 1978; Lijinsky and Andrews, 1980), although some investigators, including Venitt *et al.* (1977), who used an *Escherichia coli* foward mutation system, observed acrylonitrile mutagenicity in the absence of exogenous metabolic activation. Léonard *et al.* (1999) provided a similar assessment of the activity of acrylonitrile in *Salmonella,* concluding that base-pair and frameshift mutations were detected in a variety of *S. typhimurium* strains in the presence of metabolic activation enzymes. Responses in *Salmonella* varied quantitatively with method of exposure and the source of S9 enzymes (Lambotte-Vandepaer and Duverger-van Bogaert, 1984), and protocols that optimize the conversion of acrylonitrile to 2-cyanoethylene oxide are most likely to give a positive response (Whysner *et al.,* 1998). DNA adduct formation by 2-cyanoethylene oxide in isolated calf thymus DNA (Solomon and Segal, 1989; Solomon *et al.,* 1993; Yates *et al.,* 1993) and the weak alkylating ability of nonactivated acrylonitrile *in vitro* (Peter *et al.,* 1983) provide additional evidence of the importance of metabolic conversion to the mutagenicity of acrylonitrile.

In yeast assays conducted for the IPCS collaborative study, acrylonitrile induced gene mutations, mitotic gene conversion, and mitotic chromosome aneuploidy in the presence of metabolic activation systems (Parry, 1985).

Acrylonitrile gave positive results in IPCS *Drosophila* assays measuring induction of mitotic recombination and somatic mutations (Vogel and Nivard, 1993). Osgood *et al.* (1991) reported clearly positive results in a sex chromosome aneuploidy test in *Drosophila* females exposed to 2.7 ppm acrylonitrile vapors for up to 70 minutes. However, no induction of sex-linked recessive lethal mutations was detected in germ cells of adult male *Drosophila melanogaster* administered 420 ppm acrylonitrile by feeding for 3 days or 3,500 ppm by a single injection (Foureman *et al.,* 1994).

In the IPCS collaborative study, acrylonitrile was tested for induction of *in vitro* DNA damage (single-strand DNA breaks) and DNA repair [detected as unscheduled DNA synthesis (UDS)] (Williams, 1985; Williams *et al.,* 1985). Mostly positive results were obtained in the DNA damage tests (Bradley, 1985; Douglas *et al.,* 1985) that used alkaline gradient or sucrose sedimentation to measure single-strand DNA breaks, but results of three of four studies measuring induction of UDS were negative (Martin and Campbell, 1985; Probst and Hill, 1985; Williams, 1985; Williams *et al.,* 1985). The single positive result from the UDS test with acrylonitrile (Glauert *et al.,* 1985) came from a study that employed liquid scintillation counting of cells cultured in medium containing hydroxyurea. This method may produce a greater number of false positives than other methods of UDS detection (Williams, 1985; Williams *et al.,* 1985; Whysner *et al.,* 1998). Mixed results continue to be reported in tests for induction of UDS *in vitro* and *in vivo*. *In vivo* studies that used autoradiography for detection of UDS gave negative results regardless of cell type or method of exposure (Butterworth *et al.,* 1992; Whysner *et al.,* 1998), while studies that assessed UDS by liquid scintillation counting yielded positive results (Ahmed *et al.,* 1992, 1996; Abdel-Rachman *et al.,* 1994). Whysner *et al.* (1998) argued that liquid scintillation counting is less precise at identifying activity that is solely the result of DNA repair, and they expressed more confidence in autoradiographic detection methods. Butterworth *et al.* (1992) reported induction of UDS (measured by autoradiography) by 2-cyanoethylene oxide in human mammary epithelial cells *in vitro* but not in rat hepatocytes or spermatocytes following *in vivo* exposure. The authors suggested that this pattern of activity demonstrated a tissue-specific response.

Mutagenic activity by acrylonitrile in cultured mammalian cells was reported by a number of laboratories and most responses occurred with and without exogenous metabolic activation. Mutations were induced in human lymphoblastoid cells at the HGPRT and TK loci (Crespi *et al.,* 1985) and in L5178Y mouse lymphoma cells at the $TK^{+/-}$ locus (Amacher and Turner, 1985; Lee and Webber, 1985; Myhr *et al.,* 1985; Recio and Skopek, 1988). Further studies with the metabolite 2-cyanoethylene oxide demonstrated potent mutagenicity at the HPRT locus of human lymphoblastoid cells; both point mutations and single and multiple exon deletions were detected (Recio *et al.,* 1990).

Results of IPCS *in vitro* mammalian cell chromosomal aberration assays with acrylonitrile in human and rodent cell lines were generally positive, with and without S9, and sister chromatid exchanges were induced by acrylonitrile in several different mammalian cell lines, with and without S9 (Dean, 1985).

In contrast to the positive results obtained with acrylonitrile in numerous *in vitro* mutagenicity assays in bacteria, yeast, and mammalian cells, the majority of *in vivo* mammalian cell assays measuring a variety of endpoints gave negative results. These included tests for chromosome aberration induction in mouse (Rabello-Gay and Ahmed, 1980; Léonard *et al.,* 1981; Sharief *et al.,* 1986) and rat (Rabello-Gay and Ahmed, 1980) bone marrow micronucleus (Léonard *et al.,* 1981; Morita *et al.,* 1997) and sister chromatid exchange induction (Sharief *et al.,* 1986) in mice, and induction of dominant lethal mutations in rat (Working *et al.,* 1987) and mouse (Léonard *et al.,* 1981) sperm. Wakata *et al.* (1998) reported a small but significant increase in micronucleated erythrocytes in the bone marrow, but not the blood, of rats given 124.8 mg/kg acrylonitrile by intraperitoneal injection twice at 24-hour intervals.

The contrast between *in vitro* and *in vivo* test results with acrylonitrile in chromosomal damage assays may be due to differences in the metabolism of acrylonitrile in intact animals compared to cultured cells. In animals, less efficient metabolism of 2-cyanoethylene oxide may occur, or there may be rapid detoxification and elimination of 2-cyanoethylene oxide, thereby reducing the chance for DNA interaction.

There have been conflicting reports of elevated chromosome aberration frequencies in workers occupationally exposed to acrylonitrile. In a study of 18 workers in a polymer manufacturing plant, Thiess and Fleig (1978) found no increase in aberration frequencies among workers exposed to an estimated average atmospheric concentration of 1.5 to 5.0 ppm acrylonitrile. The average exposure duration in this study was about 15 years. In contrast, Borba *et al.* (1996) reported a significant increase in chromosomally aberrant lymphocytes in a group of 15 workers exposed to acrylonitrile in a Portuguese textile plant; no increases in sister chromatid exchanges or urine mutagenicity (measured with the Ames test) were seen in these workers. Although this study carefully assessed and controlled for other exposures and lifestyle factors that might have affected chromosomal aberration frequencies, there was no quantitative assessment of worker exposure to acrylonitrile. Additional studies of this endpoint in acrylonitrile-exposed workers are warranted. A detailed epidemiologic investigation of 46,000 infants born within 25 kilometers of an acrylonitrile manufacturing plant in Hungary during a 16-year period revealed no pattern of congenital birth defects signaling teratogenicity or germinal mutations attributable to geographic proximity to the plant (Czeizel *et al.,* 1999).

Study Rationale

In 1994, the National Cancer Institute nominated methacrylonitrile (methyl acrylonitrile) for toxicity and carcinogenicity testing by the National Toxicology Program. One of the main reasons for this nomination was methacrylonitrile's structural similarity to the known rat carcinogen, acrylonitrile. In considering this nomination, the National Institute of Environmental Health Sciences nominated acrylonitrile for toxicity and carcinogenicity studies in mice because of its extensive production and use, the high potential for human exposure, its classification as a probable human carcinogen, evidence of carcinogenicity at multiple sites in rats, and the lack of acrylonitrile carcinogenicity studies in a second animal species.

The carcinogenicity of acrylonitrile has been investigated in rats in a number of studies (USEPA, 1983; WHO, 1983; ATSDR, 1990; IARC, 1999). These studies showed that acrylonitrile is a multisite carcinogen in rats and that the target organs, brain, spinal cord, forestomach, small intestine, tongue, mammary gland, and Zymbal's gland, varied among the studies. Generally, an overlap of acrylonitrile carcinogenicity occurred in the target organs of those rats that were dosed with acrylonitrile by gavage or exposed to acrylonitrile by inhalation or in drinking water. This indicated that acrylonitrile is carcinogenic in rats and that its target organs overlapped regardless of the route of administration. Therefore, gavage administration was selected for the current 2-year study in mice.

MATERIALS AND METHODS

Procurement and Characterization of Acrylonitrile

Acrylonitrile was obtained from Aldrich Chemical Company (Milwaukee, WI) in two lots (02520DG and 00103TQ). Identity, purity, and stability analyses were conducted by the analytical chemistry laboratories, Radian Corporation (Austin, TX) and Battelle Memorial Institute (Columbus, OH), and the study laboratory (Appendix H). Reports on analyses performed in support of the acrylonitrile studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a colorless liquid, was identified as acrylonitrile by infrared, ultraviolet/visible (lot 02520DG), and proton and carbon-13 (lot 00103TQ) nuclear magnetic resonance spectroscopy and gas chromatography/mass spectroscopy (lot 02520DG). The purity of each lot was determined by elemental analyses (lot 00103TQ), Karl Fischer water analysis, and gas chromatography. Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for acrylonitrile. Karl Fischer water analysis indicated 0.18% water for lot 02520DG and 0.6% water for lot 00103TQ. Gas chromatography indicated one major peak and no impurities with areas greater than 0.1% relative to the major peak area for both lots. The overall purity of each lot was determined to be greater than 99%.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using gas chromatography. These studies indicated that there was no degradation of acrylonitrile following storage for 14 days when protected from light and air at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature, protected from light, in amber glass bottles. Stability was monitored during the studies using gas chromatography. During the studies, no degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared every 4 weeks by mixing acrylonitrile with deionized water (Table H2). Stability studies of a 0.8145 mg/mL formulation were performed by the analytical chemistry laboratory using gas chromatography. Stability was confirmed for at least 35 days for dose formulations stored in sealed glass vials with Teflon®-lined caps at temperatures up to 28° C.

Periodic analyses of the dose formulations of acrylonitrile were conducted at the study laboratory using gas chromatography. During the 14-week study, the dose formulations were analyzed at the beginning, midpoint, and end of the study (Table H3). During the 2-year study, the dose formulations were analyzed approximately every 8 to 12 weeks (Table H4). All of the dose formulations analyzed and used during the 14-week and 2-year studies were within 10% of the target concentrations.

14-Week Study

The 14-week study was conducted to evaluate the cumulative toxic effects of repeated exposure to acrylonitrile and to determine the appropriate doses to be used in the 2-year study.

Male and female $B6C3F_1$ mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY). On receipt, the mice were 4 weeks old. The animals were quarantined for 12 (males) or 13 (females) days and were 6 weeks old on the first day of the study. Before the study began, five male and five female mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At 4 weeks, serologic analyses were performed on five male and five female sentinel mice. At the end of the study, serologic analyses were performed on blood samples pooled from control and dosed mice. The serological analyses were performed according to the protocols of the NTP Sentinel Animal Program (Appendix J).

Groups of 10 male and 10 female mice were administered acrylonitrile in deionized water by gavage at doses of 0, 5, 10, 20, 40, or 60 mg acrylonitrile/kg body weight, 5 days per week, for 14 weeks. Feed and water were available *ad libitum.* Males were housed individually and females were housed five per cage. Clinical findings were recorded on day 8 and once per week thereafter. The animals were weighed initially, weekly, and at the end of the study. Details of the study design and animal maintenance are summarized in Table 1.

At the end of the 14-week study, mice were anesthetized with a mixture of carbon dioxide and oxygen, and blood was collected from the retroorbital sinus of all mice for hematology analyses. Blood samples were placed into microcollection tubes containing potassium EDTA. Erythrocyte, platelet, and leukocyte counts, hematocrit values, hemoglobin concentration, mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration were determined using a Serono-Baker System 9000 hematology analyzer (Serono-Baker Diagnostics, Allentown, PA). Differential leukocyte counts and erythrocyte and platelet morphologies were determined microscopically from blood smears stained with Wright-Giemsa stain on a Hema-Tek slide stainer (Miles Laboratory, Ames Division, Elkhart, IN). A Miller disc was used to determine reticulocyte counts from smears prepared with blood stained with new methylene blue. The parameters measured are listed in Table 1.

At the end of the 14-week study, samples were collected for sperm motility and vaginal cytology evaluations on mice that received 0, 5, 10 or 20 mg/kg (males) or 0, 10, 20, or 40 mg/kg (females) acrylonitrile. The parameters evaluated are listed in Table 1. Methods used were those described in the NTP's sperm morphology and vaginal cytology evaluations protocol (NTP, 1995). For 12 consecutive days prior to the scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Modified Tyrode's buffer was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all animals. The heart, right kidney, liver, lung, spleen, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on 0, 20 (males), 40, and 60 mg/kg mice. Table 1 lists the tissues and organs routinely examined.

2-Year Study

Study Design

Groups of 50 male and 50 female mice were administered acrylonitrile in deionized water by gavage at doses of 0, 2.5, 10, or 20 mg/kg, 5 days per week, for 104 to 105 weeks. Five male and five female mice from each group were evaluated at 2 weeks and 3, 12, and 18 months for urinalysis parameters.

Source and Specification of Animals

Male and female B6C3F$_1$ mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY) for use in the 2-year study. Mice were quarantined for 12 (males) or 11 (females) days before the beginning of the study. Five male and five female mice were randomly selected for parasite evaluation and gross observation of disease. Animals were approximately 6 weeks old at the beginning of the study. The health of the animals was monitored during the study according to the protocols of the NTP Sentinel Animal Program (Appendix J).

Animal Maintenance

Males were housed individually and females were housed five per cage. Feed and water were available *ad libitum*. Cages were changed once (males) or twice (females) weekly and rotated every 2 weeks; racks were changed and rotated every 2 weeks. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix I.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded on day 29, every 4 weeks, and at the end of the study. Body weights were recorded at the beginning of the study, approximately every 4 weeks, and at the end of the study.

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

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year study, a quality assessment pathologist evaluated slides from all tumors and from potential target organs, including the harderian gland, liver, and forestomach of males and females, and the lung, ovary, and uterus of females.

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

Urinary Metabolite Analyses

Five male and five female mice per group were randomly selected for urine collection at 2 weeks and 3, 12, and 18 months. The mice were placed individually into metabolism cages for urine collection, and urine was collected over ice during a 24-hour period, after which the mice were returned to their regular cages. The volume of urine was recorded, and urine creatinine concentrations were determined using a Hitachi 911 (Boehringer Mannheim, Indianapolis, IN) and reagents supplied by the manufacturer. Urine samples were stored frozen at -70° C or less until they were shipped to another facility for metabolite quantitation. Urinary thiocyanate was measured using a colorimetric method described by Pettigrew and Fell (1972). The parameters measured are listed in Table 1.

The acrylonitrile urinary metabolite, *N*-acetyl-S-(2-cynoethyl)-L-cysteine, was measured using liquid chromatography/mass spectrometry at an analytical chemistry laboratory (Battelle Memorial Institute). The samples were treated with acetic acid and acetonitrile to precipitate proteins and then injected onto a strong anion exchange column (Keystone Excil SAX, 4.0 × 20 mm; Keystone Scientific, Bellefonte, PA). The mobile phase was 13.14% (0.01% ornithine in water), 86.74% acetonitrile, 0.04% acetic acid, and 0.08% triethylamine, and

the system was operated isocratically at 1 mL/minute. The mass spectrometer (Perkin Elmer Sciex, Norwalk, CT) monitored the peak areas of daugter ions at m/z 86 from the m/z 215 fragment of *N*-acetyl-S-(2-cyanpropyl)-L-cysteine relative to the m/z 100 daughter ion of the m/z 229 fragment of the internal standard (*N*-acetyl-S-(2-cyanpropyl)-L-cysteine) for 0.2 seconds each. The method was validated for concentrations of 1 µg/mL and above with acceptable precision, accuracy, and recovery.

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of Acrylonitrile

14-Week Study	2-Year Study
Study Laboratory Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)
Strain and Species $B6C3F_1$ mice	$B6C3F_1$ mice
Animal Source Taconic Laboratory Animals and Services (Germantown, NY)	Taconic Laboratory Animals and Services (Germantown, NY)
Time Held Before Studies 12 (males) or 13 (females) days	12 (males) or 11 (females) days
Average Age When Studies Began 6 weeks	6 weeks
Date of First Dose 21 (males) or 22 (females) November 1995	4 (males) or 3 (females) March 1997
Duration of Dosing 5 days/week for 14 weeks	5 days/week for 104 to 105 weeks
Date of Last Dose 20 (males) or 21 (females) February 1996	2-4 March (males) or 26 February-2 March (females) 1999
Necropsy Dates 20 (males) or 21 (females) February 1996	3-5 (males) or 1-3 (females) March 1999
Average Age at Necropsy 19 weeks	110 weeks
Size of Study Groups 10 males and 10 females	50 males and 50 females
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 14-week study
Animals per Cage 1 (males) or 5 (females)	1 (males) or 5 (females)
Method of Animal Identification Tail tattoo	Tail tattoo
Diet NTP-2000 pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly	Irradiated NTP-2000 pelleted diet except during urine collection when meal feed was used (Zeigler Brothers, Inc., Gardners, PA); available *ad libitum*, changed weekly
Water Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Same as 14-week study

Table 1
Experimental Design and Materials and Methods in the Gavage Studies of Acrylonitrile

14-Week Study	2-Year Study
Cages	
Polycarbonate (Lab Products, Inc., Maywood, NJ), changed once (males) or twice (females) weekly	Same as 14-week study
Bedding	
Sani-Chips® (P.J. Murphy Forest Products Corp., Montville, NJ), changed once (males) or twice (females) weekly	Same as 14-week study, except irradiated chips were used
Cage Filters	
DuPont 2024 spun-bonded polyester (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks	Same as 14-week study
Racks	
Stainless steel (Lab Products, Inc., Maywood, NJ), rotated every 2 weeks	Same as 14-week study
Animal Room Environment	
Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Doses	
0, 5, 10, 20, 40, or 60 mg/kg in deionized water by gavage (dosing volume 10 mL/kg)	0, 2.5, 10, or 20 mg/kg in deionized water by gavage (dosing volume 10 mL/kg)
Type and Frequency of Observation	
Observed twice daily; animals were weighed initially, weekly, and at the end of the study; clinical findings were recorded on day 8 and weekly thereafter.	Observed twice daily; animals were weighed initially, approximately every 4 weeks, and at the end of the study; clinical findings were recorded on day 29, every 4 weeks, and at the end of the study.
Method of Sacrifice	
Carbon dioxide asphyxiation	Same as 14-week study
Necropsy	
Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lung, spleen, right testis, and thymus.	Necropsies were performed on all animals.
Clinical Pathology	
Hematology: Blood was collected from the retroorbital sinus of all mice surviving to the end of the study. The following parameters were measured: hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; erythrocyte and platelet morphology; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials.	***Urinary Metabolite Analyses:*** Five male and five female mice from each group were randomly selected and placed individually in metabolism cages for urine collection at 2 weeks and 3, 12, and 18 months. The urine was collected over ice during a 24-hour period, after which the mice were returned to their regular cages. The following parameters were measured: volume, creatinine, thiocyanate, and *N*-acetyl-S-(2-cyanoethyl)-L-cysteine.

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of Acrylonitrile

14-Week Study	2-Year Study
Histopathology	
Complete histopathology was performed on 0, 20 (males), 40, and 60 mg/kg mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, gallbladder, heart with aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland (females), nose, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, and urinary bladder. In addition, the stomach (forestomach) of all females surviving after day 2 was examined.	Complete histopathology was performed on all mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, gallbladder, heart with aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland (females), nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.
Sperm Motility and Vaginal Cytology	
At the end of the study, sperm samples were collected from up to 10 males in the 0, 5, 10, and 20 mg/kg groups for sperm motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, spermatid counts, and epididymal spermatozoal motility and concentration. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from up to 10 females given 0, 10, 20, or 40 mg/kg for vaginal cytology evaluations. The percentage of time spent in the various estrous cycle stages and estrous cycle length were evaluated.	None

STATISTICAL METHODS

Survival Analyses

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

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A5, B1, and B5 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A3 and B3) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A3 and B3 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, to animals that do not reach terminal sacrifice.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence.

This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal sacrifice; if the animal died prior to terminal sacrifice and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and B6C3F$_1$ mice (Portier *et al.,* 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

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

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, urinary metabolite, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1951) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973). Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across doses.

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical database must be generally similar. Until recently, the NTP historical control database consisted of animals fed NIH-07 diet. In 1995, the NTP changed the diet fed to animals used in toxicity and carcinogenesis studies conducted by the NTP. This new diet (NTP-2000) contains less protein and more fiber and fat than the NIH-07 diet previously used (Rao, 1996, 1997). This dietary change was instituted primarily to increase longevity and decrease the incidence and/or severity of some spontaneous neoplastic and nonneoplastic lesions in the rats and mice used in NTP studies. This study of acrylonitrile is one of the first in which the animals on study were fed the NTP-2000 diet. Because the incidence of some neoplastic and nonneoplastic lesions may be affected by the dietary change, use of the existing historical control database (NIH-07 diet) may not be appropriate for all neoplasm types.

Currently, the database includes 11 mouse studies by various routes in which the NTP-2000 diet was used. Based on the extensive NTP historical database using the NIH-07 diet, incidences of the vast majoity of spontaneous neoplasms are not significantly different between

control groups regardless of the route of administration. There is no reason to expect this to be different with the NTP-2000 diet. For example, control animals from dosed feed and dosed water studies are treated no differently and no differences in incidence of neoplasms are expected. Exceptions exist for some neoplasms/routes, and if comparisons are necessary for these neoplasm types, only studies with similar routes of administration will be used.

Quality Assurance Methods

The 14-week and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year study were submitted to the NTP Archives, this study was audited retrospectively by an independent quality assurance contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

Genetic Toxicology

The genetic toxicity of acrylonitrile was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium,* mutations in L51784 mouse lymphoma cells, sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells, sex-linked recessive lethal mutations and reciprocal translocations in *Drosophila melanogaster,* and increases in the frequency of micronucleated erythrocytes in mouse peripheral blood. The protocols for these studies and the results are given in Appendix C.

The genetic toxicity studies of acrylonitrile are part of a larger effort by the NTP to develop a comprehensive database that would permit a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term *in vitro* and *in vivo* genetic toxicity tests (structure-activity relationships). These short-term genetic toxicity tests were originally developed to clarify mechanisms of chemical-induced DNA damage growing out of the earlier electrophilicity/mutagenicity relationship proposed by Miller and Miller (1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). Therefore, the information obtained from these tests applies only to mutagenic carcinogens.

For mutagenic carcinogens, the combination of DNA reactivity and *Salmonella* mutagenicity is highly correlated with the induction of carcinogenicity in multiple species and genders of rodents and at multiple tissue sites (Ashby and Tennant, 1991). Data from NTP studies show that a positive response in *Salmonella* is the most predictive *in vitro* test for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) and that there is no complementarity among the *in vitro* genetic toxicity tests (Tennant *et al.,* 1987; Zeiger *et al.,* 1990). That is, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. Although other *in vitro* genetic toxicity tests correlate less well with rodent carcinogenicity compared with the *Salmonella* test, these other tests can provide useful information on the types of DNA and chromosomal effects induced by the chemical under investigation.

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

RESULTS

14-Week Study

All male and nine female mice administered 60 mg/kg acrylonitrile and eight male and three female mice administered 40 mg/kg died on the first day of the study (Table 2); one 60 mg/kg female mouse died on day 2, and one male vehicle control mouse died during week 9. Mean body weight gain of 20 mg/kg males was less than that of the vehicle control group. Clinical findings included lethargy and abnormal breathing in 40 mg/kg mice immediately after dosing. Surviving mice administered 40 mg/kg continued to show these signs of toxicity for several days until the mice appeared to develop a tolerance to acrylonitrile.

Hematology data are listed in Table D1. At week 14, decreased leukocyte counts characterized primarily by decreases in lymphocyte counts occurred in 20 mg/kg males and 40 mg/kg females. A minimal hemolytic anemia was evidenced by decreased hematocrit values, hemoglobin concentrations, and erythrocyte counts in 40 mg/kg females. Hemoglobin concentrations were minimally decreased in all dosed groups of females. Mean cell volume was also increased in 10 and 40 mg/kg females.

Heart weights of 20 mg/kg males were significantly greater than those of the vehicle controls (Table E1);

Table 2
Survival and Body Weights of Mice in the 14-Week Gavage Study of Acrylonitrile

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)
Male					
0	9/10[c]	21.3 ± 0.5	34.0 ± 1.4	12.9 ± 1.4	
5	10/10	21.2 ± 0.5	33.3 ± 0.5	12.1 ± 0.6	98
10	10/10	21.9 ± 0.3	34.1 ± 0.9	12.2 ± 0.8	100
20	10/10	21.8 ± 0.4	31.3 ± 0.9	9.5 ± 0.7*	92
40	1/10[d]	21.4 ± 0.6	34.1[e]	11.1[e]	100
60	0/10[d]	21.6 ± 0.4	—	—	—
Female					
0	10/10	18.6 ± 0.2	30.6 ± 0.9	12.0 ± 0.8	
5	10/10	18.4 ± 0.2	32.0 ± 1.0	13.5 ± 0.9	104
10	10/10	18.5 ± 0.2	29.7 ± 0.8	11.1 ± 0.9	97
20	10/10	18.7 ± 0.2	29.9 ± 1.1	11.2 ± 1.1	98
40	7/10[d]	18.7 ± 0.2	28.4 ± 1.0	9.5 ± 1.0	93
60	0/10[d]	18.5 ± 0.3	—	—	—

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test
[a] Number of animals surviving at 14 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. No final mean body weights or weight changes were calculated for groups with 100% mortality.
[c] Week of death: 9
[d] Week of death: 1
[e] No standard error was calculated due to high mortality.

organ weights of dosed groups of females were generally similar to those of the vehicle control group. The left cauda epididymis weights of 10 and 20 mg/kg males were significantly increased (Table F1); however, acrylonitrile administered at 5, 10, or 20 mg/kg for 14 weeks had no effect on sperm motility. There were no significant differences in vaginal cytology parameters between dosed and vehicle control females (Table F2).

The incidences of minimal to mild chronic active inflammation and hyperplasia in the forestomach of 40 mg/kg females were significantly greater than those in the vehicle controls (Table 3). No lesions were observed in the forestomach of mice from any other group, including the 60 mg/kg mice that died on the first day of dosing. The hyperplasia was focal, characterized by increased thickness of the stratified squamous epithelium and endophytic proliferation. The chronic active inflammation was associated with the hyperplastic changes. Two females had focal ulceration associated with the hyperplasia.

Dose Selection Rationale: Based on reduced survival in males and females and the occurrence of forestomach lesions in 40 mg/kg females, the highest acrylonitrile dose selected for the 2-year gavage study was 20 mg/kg.

Table 3
Incidences of Nonneoplastic Lesions of the Forestomach in Mice in the 14-Week Gavage Study of Acrylonitrile

	Vehicle Control	5 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg	60 mg/kg
Male						
Stomach, Forestomach[a]	10	0	0	10	2	0
Inflammation, Chronic Active[b]	0			0	0	
Ulcer	0			0	0	
Epithelium, Hyperplasia	0			0	0	
Female						
Stomach, Forestomach	10	10	10	10	7	0
Inflammation, Chronic Active	0	0	0	0	4** (1.8)[c]	
Ulcer	0	0	0	0	2 (4.0)	
Epithelium, Hyperplasia	0	0	0	0	5** (1.6)	

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

2-Year Study

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 4 and in the Kaplan-Meier survival curves (Figure 2). Survival of 20 mg/kg mice was significantly less than that of the vehicle control groups.

Body Weights and Clinical Findings

Mean body weights of 20 mg/kg males and females were generally less than those of the vehicle controls throughout most of the study; however, those of the surviving 20 mg/kg females were similar to the vehicle controls during the last 25 weeks of the study (Figure 3; Tables 5 and 6). There were no clinical findings related to acrylonitrile exposure.

Urinary Metabolite Analyses

In general, there were dose-related increases in urinary thiocyanate and *N*-acetyl-S-(2-cyanoethyl)-L-cysteine concentrations in all dosed groups of mice at 2 weeks and at 3, 12, and 18 months (Table G1; Figures 4 and 5). However, there were apparent increases in the excretion of each metabolite at 12 and 18 months compared to 2 weeks and 3 months.

Table 4
Survival of Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Male				
Animals initially in study	50	50	50	50
Accidental deaths[a]	0	2	0	0
Moribund	1	3	3	4
Natural deaths	11	3	8	32
Animals surviving to study termination	38	42	39	14
Percent probability of survival at end of study[b]	76	88	78	28
Mean survival (days)[c]	707	689	696	571
Survival analysis[d]	P<0.001	P=0.222N	P=1.000N	P<0.001
Female				
Animals initially in study	50	50	50	50
Accidental death[a]	0	0	0	1
Moribund	5	3	1	1
Natural deaths	6	15	10	25
Animals surviving to study termination	39	32	39	23[e]
Percent probability of survival at end of study	78	64	78	47
Mean survival (days)	709	691	707	606
Survival analysis	P=0.002	P=0.156	P=1.000N	P=0.001

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

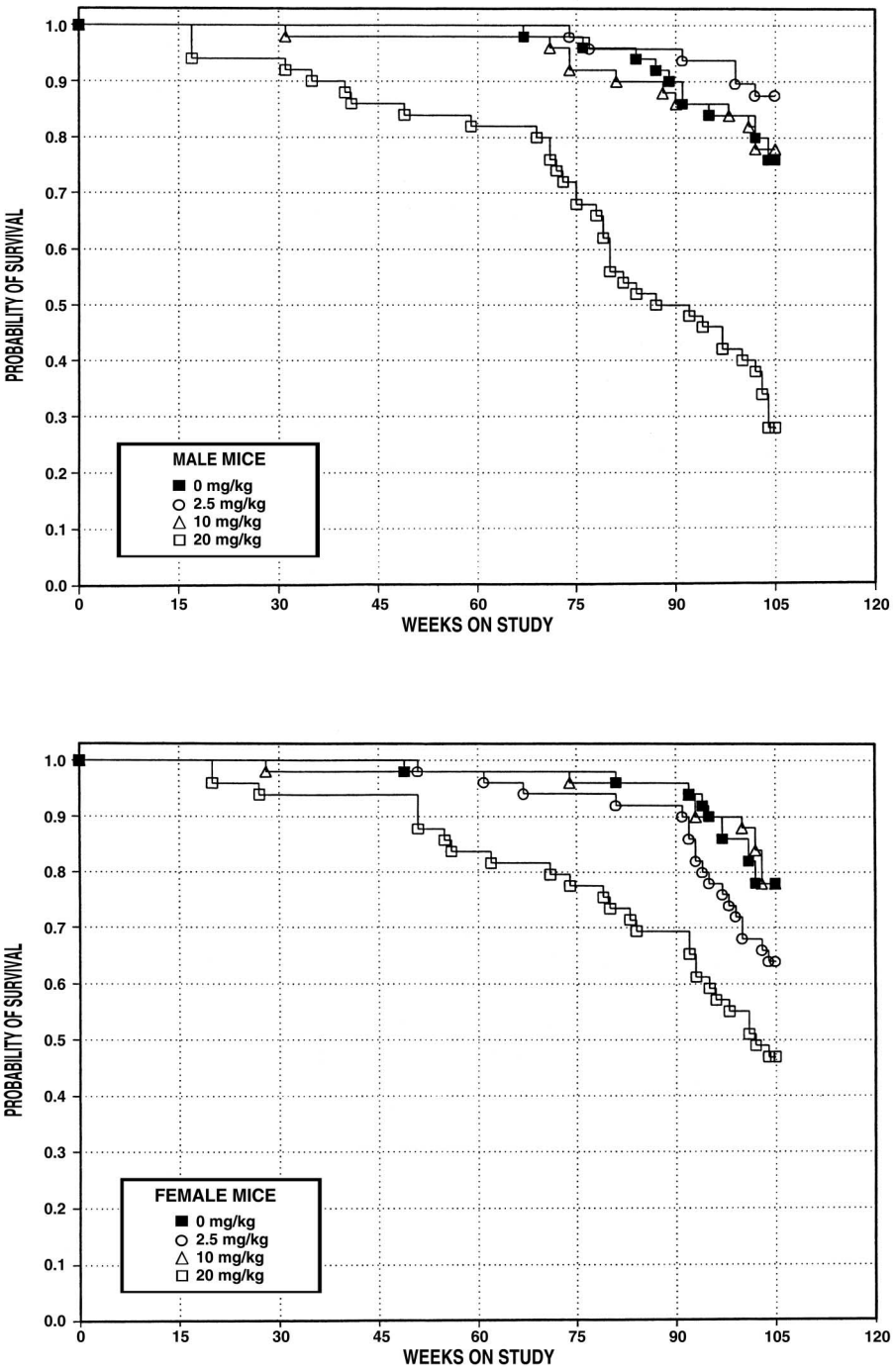
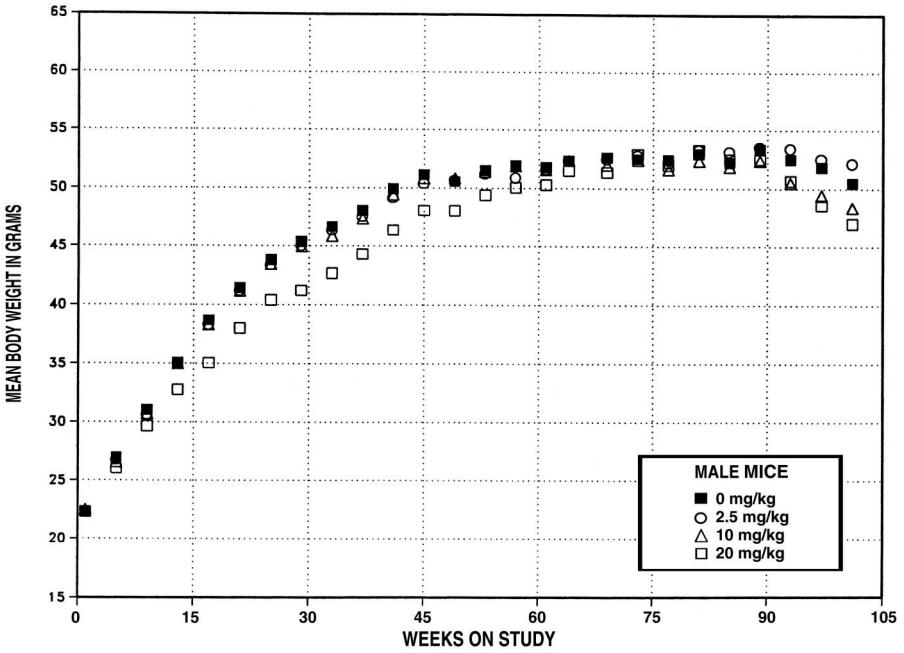


FIGURE 2
Kaplan-Meier Survival Curves for Male and Female Mice Administered Acrylonitrile by Gavage for 2 Years



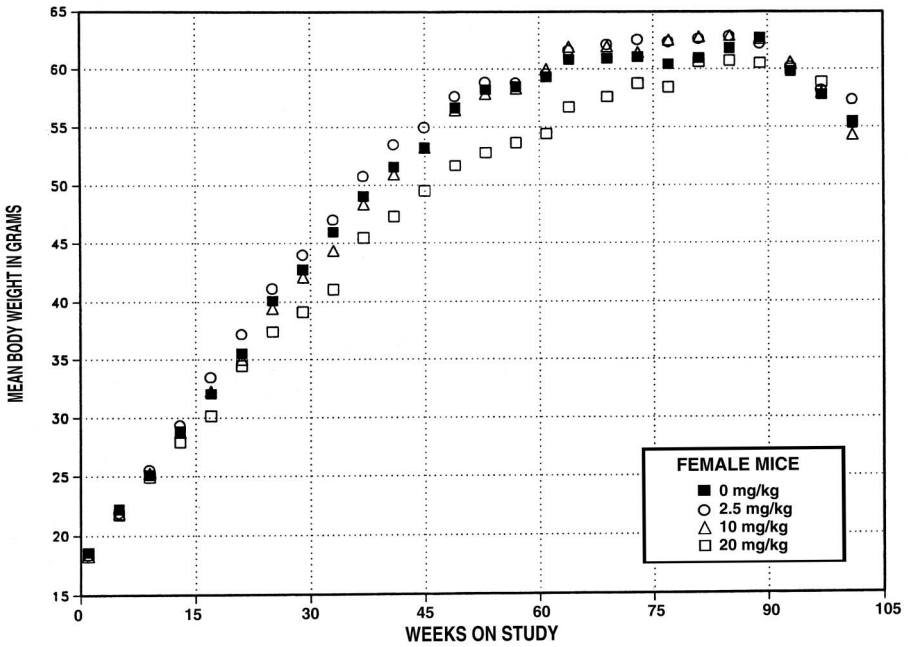


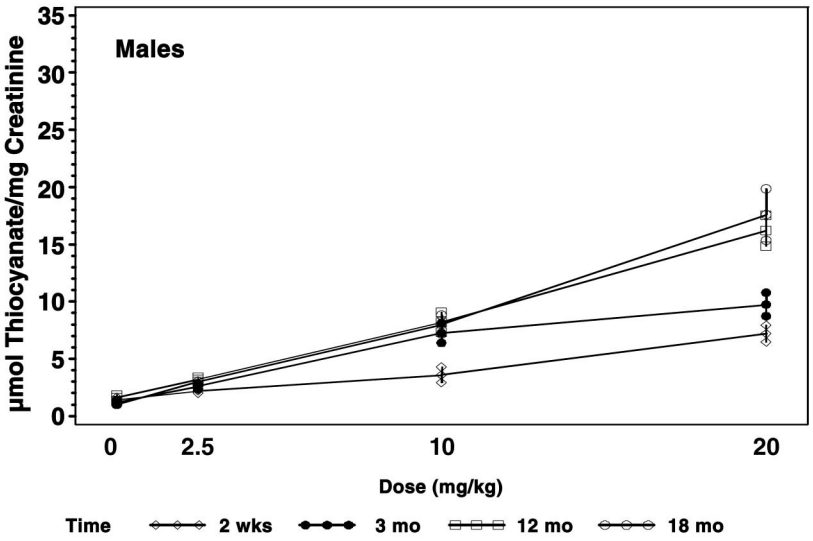
FIGURE 3
Growth Curves for Male and Female Mice Administered Acrylonitrile by Gavage for 2 Years

Table 5
Mean Body Weights and Survival of Male Mice in the 2-Year Gavage Study of Acrylonitrile

Weeks on Study	Vehicle Control		2.5 mg/kg			10 mg/kg			20 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	22.3	50	22.2	100	50	22.5	101	50	22.3	100	50
5	27.0	50	26.8	99	48	26.6	99	50	26.1	97	50
9	31.1	50	30.7	99	48	30.7	99	50	29.6	95	50
13	35.1	50	35.0	100	48	35.0	100	50	32.8	93	50
17	38.7	50	38.3	99	48	38.3	99	50	35.1	91	50
21	41.4	50	41.2	100	48	41.2	100	50	38.0	92	47
25	43.8	50	43.4	99	48	43.5	99	50	40.4	92	47
29	45.4	50	45.0	99	48	45.0	99	50	41.2	91	47
33	46.7	50	46.4	99	48	45.9	98	49	42.7	91	46
37	48.1	50	47.5	99	48	47.4	99	49	44.3	92	45
41	49.9	50	49.2	99	48	49.4	99	49	46.4	93	44
45	51.2	50	50.4	98	48	50.8	99	49	48.1	94	43
49	50.6	50	50.7	100	48	50.8	100	49	48.0	95	43
53	51.5	50	51.2	99	48	51.4	100	49	49.4	96	42
57	52.0	50	50.9	98	48	51.9	100	49	50.1	96	42
61	51.8	50	51.6	100	48	51.6	100	49	50.3	97	41
64	52.4	50	52.4	100	48	52.4	100	49	51.5	98	41
69	52.6	49	52.5	100	48	52.0	99	49	51.4	98	40
73	52.5	49	52.8	101	48	52.4	100	48	53.0	101	36
77	52.5	48	52.2	99	47	51.7	99	46	52.0	99	34
81	53.0	48	53.3	101	46	52.4	99	46	53.3	101	28
85	52.2	47	53.2	102	46	51.9	99	45	52.5	101	26
89	53.4	45	53.5	100	46	52.4	98	44	52.5	98	25
93	52.5	43	53.4	102	45	50.5	96	43	50.7	97	24
97	51.9	42	52.6	101	45	49.5	95	43	48.6	94	22
101	50.5	42	52.2	103	43	48.4	96	42	47.0	93	20
Mean for weeks											
1-13	28.9		28.7	99		28.7	99		27.7	96	
14-52	46.2		45.8	99		45.8	99		42.7	92	
53-101	52.2		52.4	100		51.4	98		50.9	98	

TABLE 6
Mean Body Weights and Survival of Female Mice in the 2-Year Gavage Study of Acrylonitrile

Weeks on Study	Vehicle Control		2.5 mg/kg			10 mg/kg			20 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	18.6	50	18.4	99	50	18.2	98	50	18.4	99	50
5	22.2	50	21.9	99	50	21.9	99	50	21.8	98	50
9	25.1	50	25.5	102	50	25.3	101	50	24.9	99	50
13	28.8	50	29.4	102	50	28.8	100	50	27.9	97	50
17	32.1	50	33.5	104	50	32.3	101	50	30.2	94	49
21	35.5	50	37.2	105	50	35.0	99	50	34.5	97	47
25	40.1	50	41.1	103	50	39.4	98	50	37.4	93	47
29	42.7	50	44.0	103	50	42.1	99	49	39.1	92	46
33	46.0	50	47.0	102	50	44.4	97	49	41.1	89	46
37	49.0	50	50.8	104	50	48.3	99	49	45.5	93	46
41	51.6	50	53.5	104	50	50.9	99	49	47.3	92	46
45	53.3	50	55.0	103	50	53.3	100	49	49.5	93	46
49	56.7	50	57.6	102	50	56.4	100	49	51.7	91	46
53	58.2	49	58.8	101	49	57.8	99	49	52.8	91	43
57	58.5	49	58.8	101	49	58.3	100	49	53.7	92	41
61	59.3	49	59.6	101	49	60.0	101	49	54.5	92	41
64	60.8	49	61.6	101	48	61.9	102	49	56.7	93	40
69	60.9	49	62.1	102	47	62.0	102	49	57.6	95	40
73	61.1	49	62.6	103	47	61.5	101	49	58.7	96	39
77	60.4	49	62.4	103	47	62.5	104	48	58.4	97	38
81	61.0	49	62.6	103	47	62.8	103	48	60.6	99	36
85	61.8	48	62.9	102	46	62.9	102	48	60.7	98	34
89	62.7	48	62.2	99	46	62.7	100	48	60.5	97	34
93	59.8	47	60.2	101	43	60.6	101	47	60.0	100	32
97	57.8	44	58.2	101	38	58.0	100	45	58.9	102	28
101	55.3	42	57.4	104	34	54.4	98	44	55.4	100	27
Mean for weeks											
1-13	23.7		23.8	100		23.6	100		23.3	98	
14-52	45.2		46.6	103		44.7	99		41.8	92	
53-101	59.8		60.7	102		60.4	101		57.6	96	



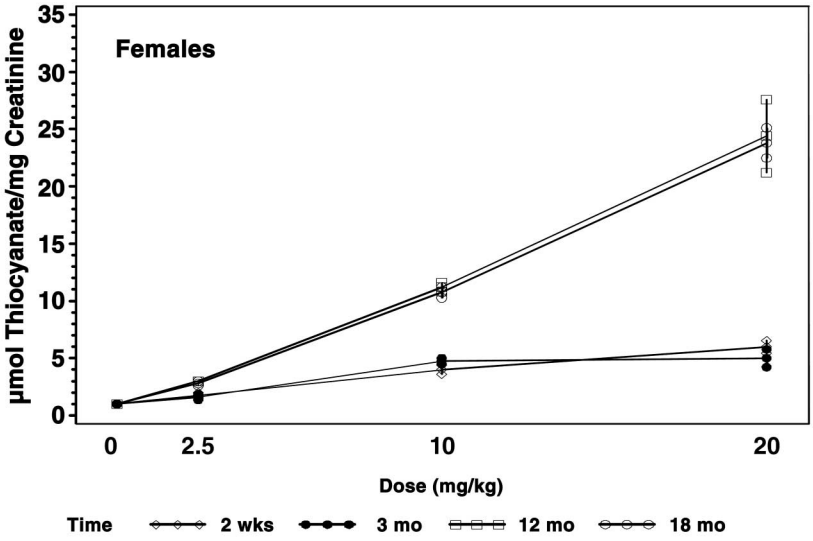


FIGURE 4
Urinary Thiocyanate Concentrations in Male and Female Mice at 2 Weeks and at 3, 12, and 18 Months (data are presented as mean ± standard error)

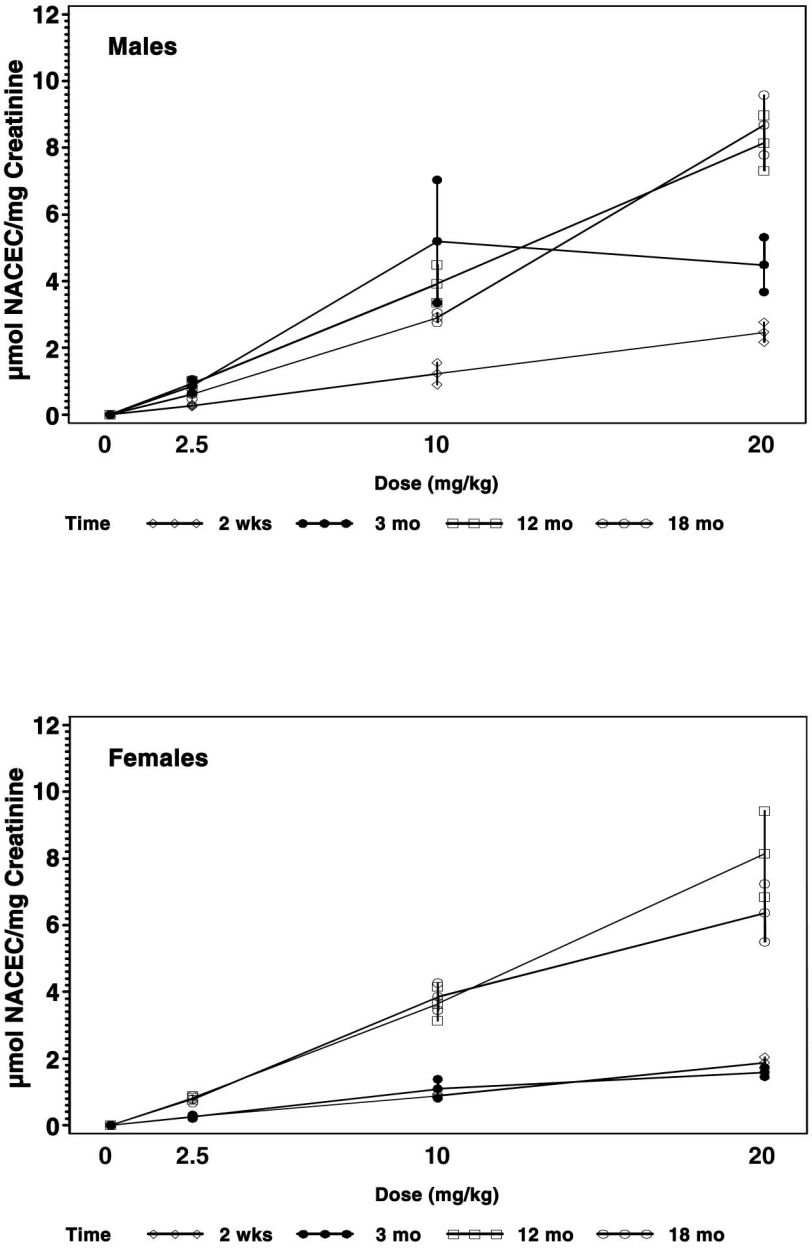


FIGURE 5
***N*-Acetyl-S-(2-cyanoethyl)-L-cysteine (NACEC) Concentrations in Male and Female Mice at 2 Weeks and at 3, 12, and 18 Months**
(data are presented as mean ± standard error)

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and nonneoplastic lesions of the forestomach, harderian gland, ovary, lung, and liver. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male mice and Appendix B for female mice.

Forestomach: The incidences of squamous cell papilloma, squamous cell carcinoma, and squamous cell papilloma or carcinoma (combined) of the forestomach occurred with positive trends in males and females, and the incidences in 10 and 20 mg/kg mice were generally significantly greater than those in the vehicle controls (Tables 7, A3, and B3). The incidences of these lesions in all dosed groups exceeded the historical ranges in controls (all routes) given NTP-2000 diet and in water gavage controls given NIH-07 diet (Tables 7, A4a, and B4a). Squamous cell papillomas consisted of pedunculated masses protruding into the lumen. Papillomas were composed of numerous, branching, finger-like projections arising from a stalk (Plate 1). The projections were covered by one to many layers of squamous epithelium. Squamous cell carcinomas were well differentiated with some keratin pearl formation (Plate 2); however, some had evidence of submucosal invasion. Poorly differentiated squamous cell carcinomas were composed of spindle-shaped cells without keratinization. Some squamous cell carcinomas metastasized, primarily to the liver but also to the pancreas, spleen, kidney, lung, mesenteric lymph nodes, prostate gland, and adrenal gland.

The incidences of mild focal or multifocal epithelial hyperplasia (combined) in 20 mg/kg males and females were significantly greater than those in the vehicle controls (Tables 7, A5, and B5); the incidence of mild diffuse or focal hyperkeratosis (combined) in 20 mg/kg males was significantly increased. Hyperplasia was characterized by thickened, orderly, and maturing epithelium. The thickened epithelium formed either endophytic pegs or closely apposed undulating folds (Plate 3). The hyperplastic lesions were often accompanied by focal, and occasionally diffuse, hyperkeratosis and were occasionally associated with chronic active inflammation. Hyperkeratosis without hyperplasia was rarely observed. Sporadic cases of multifocal ulcerations of the forestomach were observed in 2.5 and 10 mg/kg males; the ulcerations were generally accompanied by focal chronic active inflammation.

Harderian Gland: The incidences of harderian gland adenoma and adenoma or carcinoma (combined) occurred with positive trends in males and females. The incidences in 2.5 mg/kg males and in 10 and 20 mg/kg males and females were significantly increased and exceeded the historical ranges in controls (all routes) given NTP-2000 diet and in water gavage controls given NIH-07 diet (Tables 8, A3, A4b, B3, and B4b). Adenomas were characterized by loss of alveolar structure and at least minimal compression of the surrounding tissue. These neoplasms were usually unilateral but were occasionally bilateral (Plate 4). Carcinomas generally resembled adenomas, but featured localized invasion of the structures adjacent to the gland and/or distant metastases (Plate 5).

The incidences of harderian gland hyperplasia in dosed groups of males and 10 and 20 mg/kg females were greater than those in the vehicle controls, and the incidence in 10 mg/kg males was significantly increased (Tables 8, A5, and B5). Hyperplasia was focal, without compression of the surrounding alveoli, and the cells tended to be tinctorially distinct and often larger than the normal surrounding cells. Due to the increased number of cells, the acinar walls appeared folded (Plate 6). Decreased incidences of mononuclear cell infiltrates in dosed males and females were attributed to replacement of the normal gland by the presence of neoplasms.

The harderian gland is a secretory gland located medial and posterior to the globe of the eye. Large proliferative lesions of the harderian gland result in grossly observable compression, protrusion (proptosis), and collapse of the eye. Secondary changes were noted in those eyes that were compressed by the harderian gland neoplasms (Tables A5 and B5).

TABLE 7
Incidences of Neoplasms and Nonneoplastic Lesions of the Forestomach in Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Male				
Number Necropsied	50	50	50	50
Hyperkeratosis, Diffuse or Focal[a]	2 (2.5)[b]	3 (2.0)	7 (1.7)	12** (1.8)
Epithelium, Hyperplasia, Focal	2 (3.0)	4 (2.3)	8* (2.0)	9** (1.9)
Ulcer, Multifocal	0	2 (2.5)	1 (2.0)	0
Squamous Cell Papilloma, Multiple	0	0	2	9**
Squamous Cell Papilloma (includes multiple)	3	4	19**	25**
Squamous Cell Carcinoma	0	0	8**	9**
Squamous Cell Papilloma or Carcinoma[c]				
Overall rate[d]	3/50 (6%)	4/50 (8%)	26/50 (52%)	32/50 (64%)
Adjusted rate[e]	6.4%	8.7%	55.7%	83.3%
Terminal rate[f]	2/38 (5%)	4/42 (10%)	21/39 (54%)	12/14 (86%)
First incidence (days)	464	730 (T)	515	410
Poly-3 test[g]	P<0.001	P=0.489	P<0.001	P<0.001
Female				
Number Necropsied	50	50	50	50
Hyperkeratosis, Diffuse or Focal	2 (1.5)	1 (2.0)	2 (2.0)	4 (2.0)
Epithelium, Hyperplasia, Focal or Multifocal	2 (1.5)	2 (3.0)	5 (1.8)	7* (1.6)
Ulcer, Multifocal	1 (2.0)	1 (2.0)	0	0
Squamous Cell Papilloma, Multiple	1	0	4	9**
Squamous Cell Papilloma (includes multiple)	3	6	24**	19**
Squamous Cell Carcinoma	0	1	1	11**
Squamous Cell Papilloma or Carcinoma[h]				
Overall rate	3/50 (6%)	7/50 (14%)	25/50 (50%)	29/50 (58%)
Adjusted rate	6.4%	15.7%	52.7%	75.4%
Terminal rate	3/39 (8%)	5/32 (16%)	22/39 (56%)	17/23 (74%)
First incidence (days)	729 (T)	665	639	513
Poly-3 test	P<0.001	P=0.136	P<0.001	P<0.001

(T)Terminal sacrifice
* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year studies with controls given NTP-2000 diet (mean ± standard deviation): 11/659 (2.0% ± 2.0%), range 0%-6%; with water gavage controls given NIH-07 diet: 0/50
[d] Number of animals with neoplasm per number of animals necropsied
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h] Historical incidence for NTP-2000 diet: 10/659 (1.6% ± 1.9%), range 0%-6%; for NIH-07 diet: 0/51

TABLE 8
Incidences of Neoplasms and Nonneoplastic Lesions of the Harderian Gland in Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Male				
Harderian Gland[a]	50	50	50	50
Hyperplasia[b]	1 (2.0)[c]	4 (2.3)	7* (3.4)	4 (2.3)
Infiltration Cellular, Mononuclear Cell	33 (1.0)	25 (1.0)	26 (1.0)	14** (1.0)
Adenoma, Bilateral	0	0	9**	6**
Adenoma (includes bilateral)	5	16**	24**	27**
Carcinoma	1	1	4	3
Adenoma or Carcinoma[d]				
Overall rate[e]	6/50 (12%)	16/50 (32%)	27/50 (54%)	30/50 (60%)
Adjusted rate[f]	13.0%	34.1%	55.6%	81.3%
Terminal rate[g]	6/38 (16%)	13/42 (31%)	19/39 (49%)	12/14 (86%)
First incidence (days)	730 (T)	518	497	477
Poly-3 test[h]	P<0.001	P=0.014	P<0.001	P<0.001
Female				
Harderian Gland	50	50	50	50
Hyperplasia, Bilateral	0	0	0	1
Hyperplasia (includes bilateral)	5 (3.0)	4 (3.3)	6 (2.2)	8 (3.5)
Infiltration Cellular, Mononuclear Cell	40 (1.1)	30* (1.1)	33 (1.2)	23** (1.0)
Adenoma, Bilateral	2	0	4	4
Adenoma (includes bilateral)	10	10	25**	23**
Carcinoma	1	0	3	2
Adenoma or Carcinoma[i]				
Overall rate	11/50 (22%)	10/50 (20%)	26/50 (52%)	25/50 (50%)
Adjusted rate	23.0%	22.3%	54.5%	67.0%
Terminal rate	10/39 (26%)	8/32 (25%)	23/39 (59%)	16/23 (70%)
First incidence (days)	338	563	639	547
Poly-3 test	P<0.001	P=0.568N	P<0.001	P<0.001

(T) Terminal sacrifice
* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
** $P \leq 0.01$
[a] Number of animals necropsied
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Historical incidence for 2-year studies with controls given NTP-2000 diet (mean ± standard deviation): 57/659 (8.8% ± 4.1%), range 2%-16%; with water gavage controls given NIH-07 diet: 3/50 (6.0%)
[e] Number of animals with neoplasm per number of animals necropsied
[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A lower incidence in a dosed group is indicated by **N**.
[i] Historical incidence for NTP-2000 diet: 50/659 (7.5% ± 5.7%), range 0%-22%; for NIH-07 diet: 3/51 (6.0%)

Ovary: The incidence of benign or malignant granulosa cell tumor (combined) in the ovary of 10 mg/kg females was greater than that in the vehicle control group (Tables 9 and B3). The incidence in this group exceeded the historical range in controls (all routes) given NTP-2000 diet, in controls (various routes) given NIH-07 diet, and in corn oil gavage controls given NIH-07 diet (Table B4c). One malignant granulosa cell tumor invaded the surrounding fat. Granulosa cell tumors are sex-cord stromal tumors derived from neoplastic transformation of the mesodermal follicular stem cells in the adult ovary. The criteria used to define the benign granulosa cell tumors included the presence of varying sized follicles that compressed adjacent tissue or completely effaced the ovary (Plate 7). The follicles were composed of round to cuboidal cells arranged on a delicate basement membrane, and the cells often resembled granulosa cells of normal follicles. Granulosa cell tumors are the most common chemically induced ovarian neoplasms in NTP 2-year carcinogenicity studies.

The incidences of atrophy and cyst in the ovary of 2.5 (cyst only), 10, and 20 mg/kg females were significantly increased (Tables 9 and B5); in dosed females, the severity of atrophy was marked and the severity of cyst was mild. Atrophy was characterized by a partial to complete lack of histologically evident follicular and corpus luteum development and a predominance of interstitial tissue (Plates 8 and 9). Cysts were of follicular, epithelial inclusion, rete, or paraovarian types, and they were grouped under a common definition of cyst for statistical analysis.

There were decreased incidences of cystadenoma in the ovary of dosed females; however, the incidence in the vehicle controls was higher than in any other controls given NTP-2000 diet and exceeded the historical range in water gavage controls given NIH-07 diet (Tables 9, B3, and B4c).

Lung: The incidence of alveolar/bronchiolar adenoma or carcinoma (combined) in 10 mg/kg females was significantly greater than that in the vehicle controls (Tables 10 and B3). The incidences of alveolar/bronchiolar carcinoma in dosed groups and the incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in 10 and 20 mg/kg females generally exceeded the historical ranges for controls (all routes) given NTP-2000 diet and for water gavage controls or feed controls given NIH-07 diet (Table B4d). The lower incidences of primary alveolar/bronchiolar adenomas and carcinomas in 20 mg/kg females may have been related to reduced survival in this group. The adenomas were distinct and compressing nodules that distorted the underlying alveolar structure, and the epithelial arrangement was irregular, papillary, glandular, or had solid patterns of cuboidal to columnar epithelium (Plate 10). In contrast to the adenomas, the alveolar/bronchiolar carcinomas (Plate 11) showed heterogenic growth patterns, cellular anaplasia with pleomorphism, nuclear atypia, localized invasiveness, or intrapulmonary or distant metastases. Incidences of alveolar epithelial hyperplasia were similar in vehicle control and dosed groups of females (Tables 10 and B5). The incidences of alveolar/bronchiolar neoplasms in male mice were similar to those in the vehicle controls (Tables 10 and A1).

Liver: The incidence of hepatocellular adenoma in 2.5 mg/kg males was significantly greater than that in the vehicle control group (vehicle control, 23/50; 2.5 mg/kg, 32/50; 10 mg/kg, 29/50; 20 mg/kg, 14/50; Table A3), and the incidence exceeded the historical ranges in controls (all routes) given NTP-2000 diet [195/659 (30.4% ± 8.9%), range 12%-46%] or water gavage controls given NIH-07 diet (26/50) (Table A4c). The incidences of hepatocellular adenoma in 10 mg/kg males and of hepatocellular adenoma or carcinoma (combined) (32/50, 36/50, 37/50, 17/50) in vehicle control, 2.5, and 10 mg/kg males also exceeded the historical ranges; however, the incidences of these neoplasms in these groups were not significantly increased or dose related.

TABLE 9
Incidences of Neoplasms and Nonneoplastic Lesions of the Ovary in Female Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Number Examined Microscopically	50	50	50	50
Atrophy[a]	6 (3.0)[b]	8 (3.9)	45** (4.0)	40** (4.0)
Cyst	12 (2.3)	20* (2.3)	27** (2.1)	19* (2.1)
Cystadenoma[c]				
Overall rate[d]	8/50 (16%)	4/50 (8%)	2/50 (4%)	3/50 (6%)
Adjusted rate[e]	17.0%	9.1%	4.3%	8.5%
Terminal rate[f]	7/39 (18%)	4/32 (13%)	2/39 (5%)	2/23 (9%)
First incidence (days)	659	729 (T)	729 (T)	704
Poly-3 test[g]	P=0.112N	P=0.211N	P=0.046N	P=0.219N
Benign Granulosa Cell Tumor	0	0	3	1
Malignant Granulosa Cell Tumor	0	0	1	0
Benign or Malignant Granulosa Cell Tumor[h]				
Overall rate	0/50 (0%)	0/50 (0%)	4/50 (8%)	1/50 (2%)
Adjusted rate	0.0%	0.0%	8.5%	2.8%
Terminal rate	0/39 (0%)	0/32 (0%)	4/39 (10%)	1/23 (4%)
First incidence (days)	—[i]	—[j]	729 (T)	729 (T)
Poly-3 test	P=0.090	—	P=0.061	P=0.444

(T) Terminal sacrifice
* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year studies with controls given NTP-2000 diet (mean ± standard deviation): 24/626 (3.9% ± 4.4%), range 0%-16%; with water gavage controls given NIH-07 diet: 3/51; with corn oil gavage controls given NIH-07 diet: 5/453 (1.1% ± 1.4%), range 0%-4%
[d] Number of animals with neoplasm per number of animals with ovary examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A negative trend or lower incidence in a dosed group is indicated by **N**.
[h] Historical incidence for NTP-2000 diet: 5/626 (0.7% ± 1.0%), range 0%-3%; for NIH-07 diet (water gavage): 0/51; for NIH-07 diet (corn oil gavage): 5/453 (1.1% ± 1.5%), range 0%-4%
[i] Not applicable; no neoplasms in animal group
[j] Value of statistic cannot be computed.

TABLE 10
Incidences of Alveolar/bronchiolar Neoplasms and Nonneoplastic Lesions of the Lung in Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Male				
Number Examined Microscopically	50	50	50	50
Alveolar Epithelium, Hyperplasia[a]	4 (2.0)[b]	3 (3.0)	5 (2.4)	6 (1.7)
Alveolar/bronchiolar Adenoma	10	9	4	8
Alveolar/bronchiolar Carcinoma	4	2	5	2
Female				
Number Examined Microscopically	50	50	50	50
Alveolar Epithelium, Hyperplasia	2 (3.5)	2 (1.5)	4 (2.0)	0
Alveolar/bronchiolar Adenoma	4	1	8	5
Alveolar/bronchiolar Carcinoma[c]	2	5	6	4
Alveolar/bronchiolar Adenoma or Carcinoma[d]				
Overall rate[e]	6/50 (12%)	6/50 (12%)	14/50 (28%)	9/50 (18%)
Adjusted rate[f]	12.7%	13.3%	29.3%	25.3%
Terminal rate[g]	3/39 (8%)	1/32 (3%)	10/39 (26%)	7/23 (30%)
First incidence (days)	677	633	639	667
Poly-3 test[h]	P=0.029	P=0.588	P=0.039	P=0.121

[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year studies with controls given NTP-2000 diet (mean ± standard deviation): 17/654 (2.3% ± 2.0%), range 0%-6%; with water gavage controls given NIH-07 diet: 1/51; with feed controls given NIH-07 diet: 31/952 (3.2% ± 3.1%), range 0%-8%
[d] Historical incidence for NTP-2000 diet: 53/654 (7.6% ± 4.7%), range 0%-12%; for NIH-07 diet (water gavage): 4/51; for NIH-07 diet (feed): 81/952 (8.5% ± 3.6%), range 2%-12%
[e] Number of animals with neoplasm per number of animals with organ examined microscopically
[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.

Genetic Toxicology

Acrylonitrile (100 to 10,000 µg/plate) was mutagenic in *Salmonella typhimurium* strain TA100 in the presence of hamster liver S9 enzymes (Table C1; Zeiger and Haworth, 1985); it was also mutagenic in strain TA1535 with rat and hamster S9. It was not mutagenic in strain TA97 or TA98, with or without S9. Acrylonitrile induced mutations in mouse lymphoma L5178Y cells at concentrations of 12.5 nL/mL and higher in the absence of S9 (Table C2; Myhr *et al.,* 1985). In cultured Chinese hamster ovary cells, acrylonitrile induced sister chromatid exchanges with and without S9 (Table C3; Gulati *et al.,* 1985); chromosomal aberrations were significantly increased only in the presence of S9 (Table C4; Gulati *et al.,* 1985). No induction of sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* was observed when 415 ppm acrylonitrile was administered in feed with sucrose or 3,475 ppm in saline was administered by injection (Table C5; Foureman *et al.,* 1994). Despite the results of the statistical analysis of the data (P<0.001), the frequency of lethal mutations in flies treated by injection (0.10%) was insufficient for a positive call. The control frequency in the injection test was unusually low, and comparison of this extreme value with the low level of lethals observed in the treated flies produced the low P value in the injection experiment. Results of the reciprocal translocation test were also negative (Table C6).

In contrast to the induction of chromosomal damage in mammalian cells *in vitro*, no increase in the frequency of micronucleated normochromatic erythrocytes was observed in peripheral blood samples from male or female mice administered acrylonitrile by gavage for 14 weeks (Table C7).

Acrylonitrile induced genetic damage *in vitro* in bacterial and mammalian cells, but *in vivo* tests in *D. melanogaster* and mice were negative.

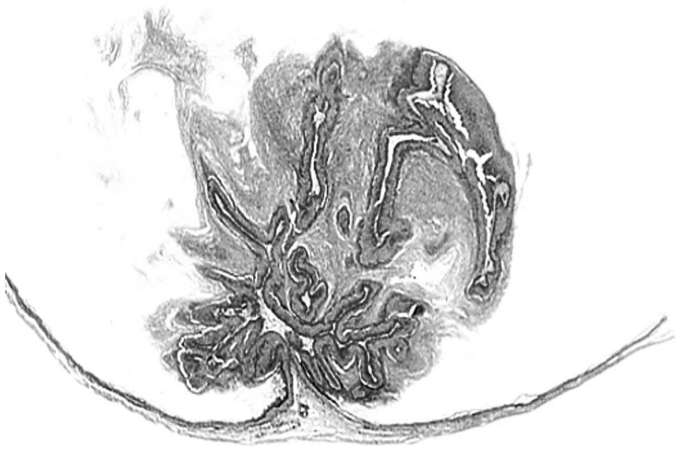


PLATE 1
Squamous cell papilloma in the forestomach of a male B6C3F$_1$ mouse given 20 mg acrylonitrile/kg body weight per day by gavage for 2 years. Note the central stalk with secondary branches. H&E; 5×

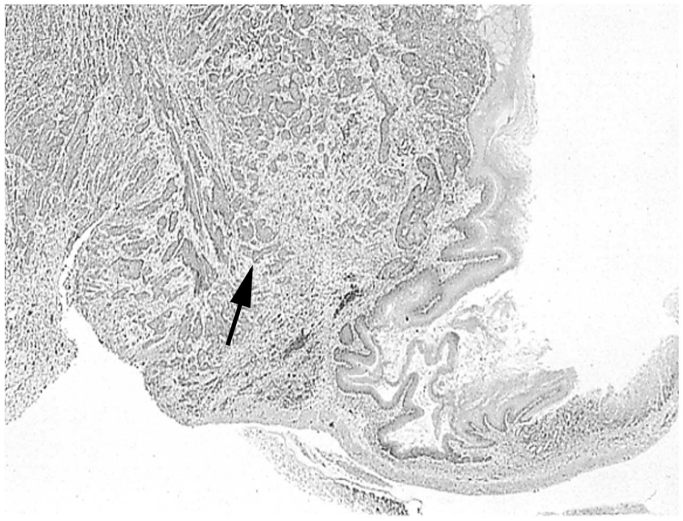


PLATE 2
Squamous cell carcinoma in the forestomach of a male B6C3F$_1$ mouse given 20 mg acrylonitrile/kg body weight per day by gavage for 2 years. Note (arrow) the invasion of neoplastic cells into the submucosa. H&E; 5×

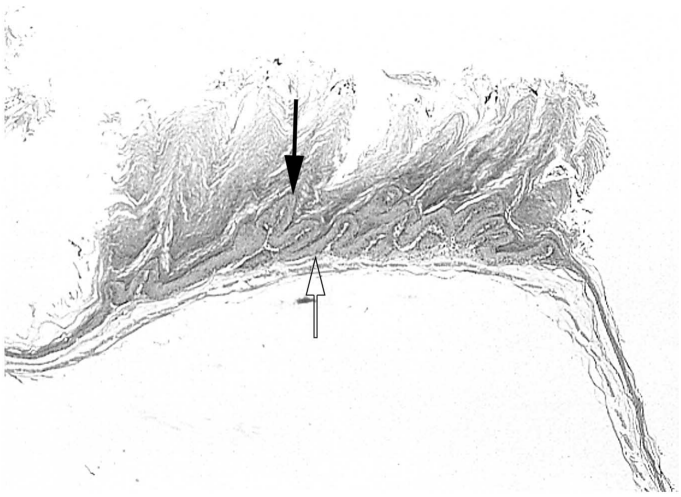


PLATE 3
Squamous cell hyperplasia in the forestomach of a male B6C3F$_1$ mouse given 20 mg acrylonitrile/kg body weight per day by gavage for 2 years. Note the thickened epithelium forming endophytic pegs (open arrow). Dark arrow indicates undulating folds. H&E; 10×

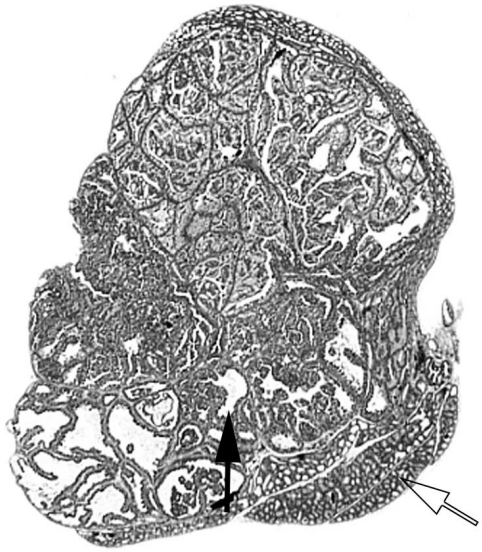


PLATE 4
Adenoma in the harderian gland of a male B6C3F$_1$ mouse given 20 mg acrylonitrile/kg body weight per day by gavage for 2 years. Note the distinct nodule compressing the surrounding alveoli. Dark arrow-adenoma, open arrow-normal harderian gland tissue. H&E; 3.3×

PLATE 5
Carcinoma in the harderian gland of a male B6C3F$_1$ mouse given 20 mg acrylonitrile/kg body weight per day by gavage for 2 years. Note the carcinoma (arrows) adjacent to the gland (N=normal gland). H&E; 8×

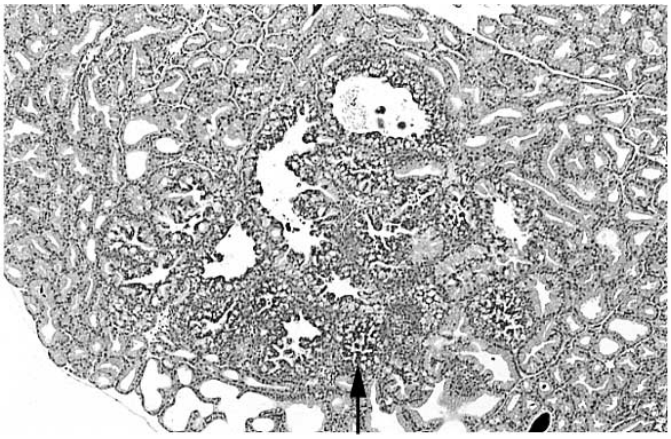


PLATE 6
Marked hyperplasia in the harderian gland of a male B6C3F$_1$ mouse given 20 mg acrylonitrile/kg body weight per day by gavage for 2 years. Note the focus of tinctorially distinct cells (arrow) with no evidence of compression of the surrounding alveoli. H&E; 16×

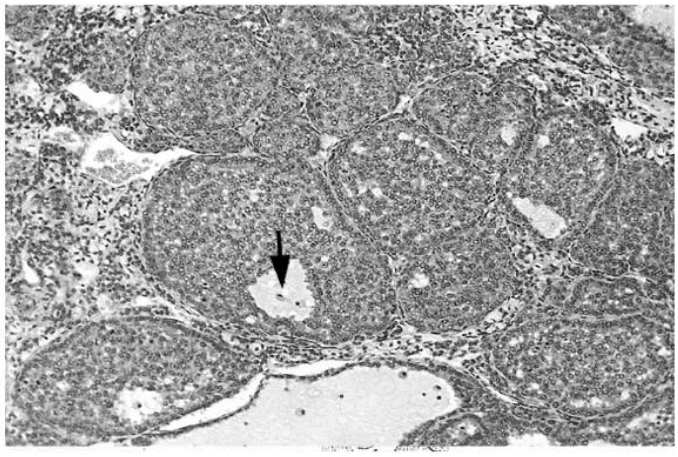


PLATE 7
Granulosa cell tumor in the ovary of a female B6C3F$_1$ mouse given 20 mg acrylonitrile/kg body weight per day by gavage for 2 years. The tumor cells, which have scanty cytoplasm, are forming varying sized pseudofollicular structures. The characteristic Call-Exner bodies (cell-free area, see arrow) are also present. H&E; 10×

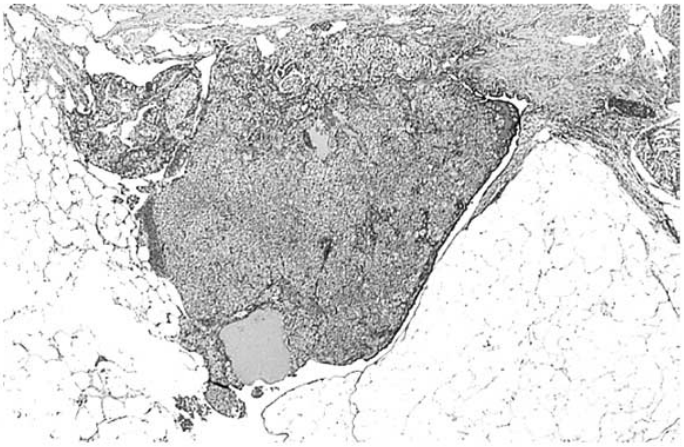


PLATE 8
Severe atrophy in the ovary of a female B6C3F$_1$ mouse given 20 mg acrylonitrile/kg body weight per day by gavage for 2 years. Note the absence of follicles and corpora lutea and a predominance of interstitial tissue. H&E; 10×

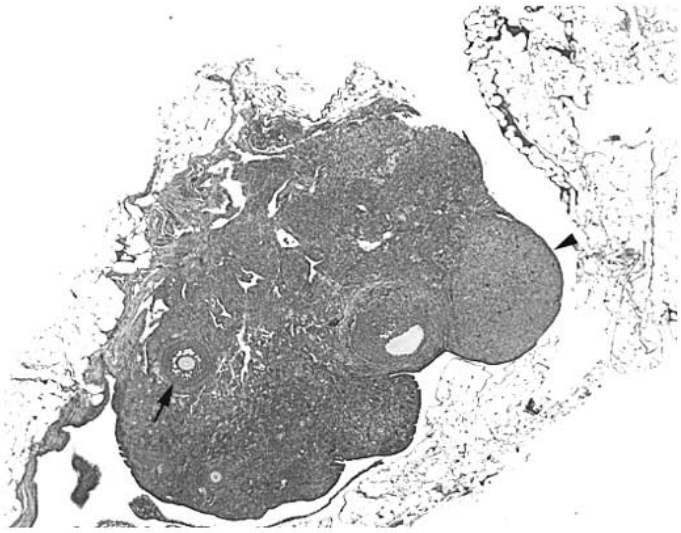


PLATE 9
Ovary of a vehicle control female B6C3F$_1$ mouse from the 2-year study of acrylonitrile. Note presence of follicle (arrow) and corpus luteum (arrowhead). H&E; 10×

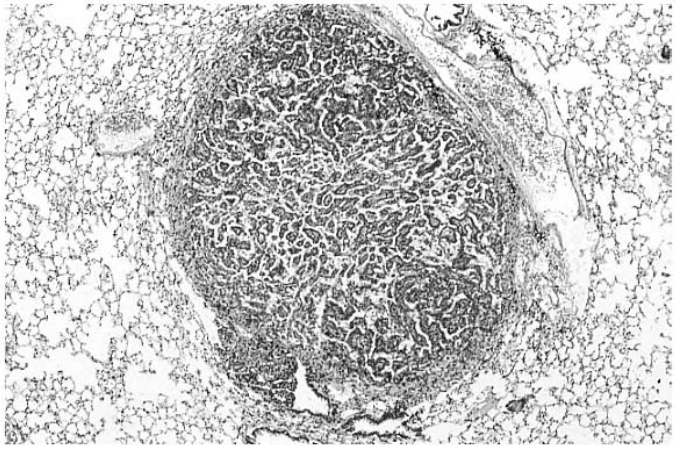


PLATE 10
Alveolar/bronchiolar adenoma in a female B6C3F$_1$ mouse given 20 mg acrylonitrile/kg body weight per day by gavage for 2 years. Note a distinct and compressing nodule distorting the underlying alveolar structure. H&E; 13.2×

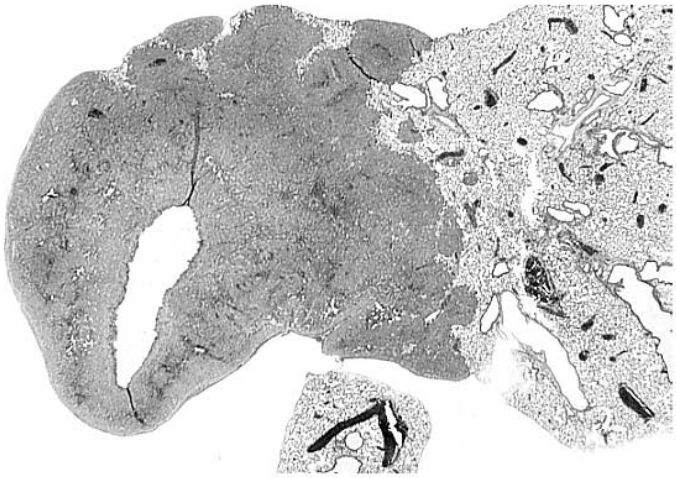


PLATE 11
Alveolar/bronchiolar carcinoma in a female B6C3F$_1$ mouse given 20 mg acrylonitrile/kg body weight per day by gavage for 2 years. H&E; 25×

DISCUSSION AND CONCLUSIONS

Acrylonitrile was nominated for toxicity and carcinogenicity studies in mice because of its extensive production and use, its high potential for human exposure, its classification as a probable human carcinogen, evidence of its carcinogenicity at multiple sites in rats, and the lack of carcinogenicity studies in a second animal species.

Long-term gavage, inhalation, and drinking water studies demonstrated that acrylonitrile is a multisite carcinogen in rats (USEPA, 1983; WHO, 1983; ATSDR, 1990; IARC, 1999). Exposure of male Sprague-Dawley rats to 0, 20, 100, or 500 ppm acrylonitrile in drinking water for 2 years resulted in significant increases in the incidences of Zymbal's gland tumors and of forestomach tumors in the 500 ppm group (Gallagher *et al.,* 1988). In a second drinking water study, Fischer 344 rats were exposed to 0, 100, or 500 ppm acrylonitrile; significant increases in the incidences of brain and spinal cord tumors were observed (Bigner *et al.,* 1986). The incidences of Zymbal's gland, forestomach, and skin tumors were also increased in acrylonitrile-treated rats. Exposure to acrylonitrile by inhalation (Maltoni *et al.,* 1988) or gavage (Maltoni *et al.,* 1977) also increased the incidences of tumors of the forestomach, mammary gland, brain, and Zymbal's gland in Sprague-Dawley rats. To date, no studies that assessed the carcinogenicity of acrylonitrile in any other animal species are available in the literature.

Epidemiology studies that were available at the time of the 1994 nomination indicated that acrylonitrile may cause cancer in humans. Based on the assumption that there was sufficient evidence of acrylonitrile carcinogenicity in rats and limited evidence in humans, IARC (1987) placed acrylonitrile in Group 2A (Probable Human Carcinogen), and the USEPA (1987) placed it in Group B1 (Probable Human Carcinogen). However, in light of newer and generally inconclusive epidemiology studies, IARC (1999) downgraded acrylonitrile to a Group 2B carcinogen (Possible Human Carcinogen). In the *9th Report on Carcinogens* (NTP, 2001), acrylonitrile is listed as "reasonably anticipated to be a human carcinogen."

In the current 14-week gavage study, male and female $B6C3F_1$ mice were administered 0, 5, 10, 20, 40, or 60 mg acrylonitrile/kg body weight, 5 days per week. All but two males that received 40 or 60 mg/kg died or were euthanized on the first day of the study. Nine 60 mg/kg females and three 40 mg/kg females died or were euthanized on the first day of the study. The mean body weight gain of 20 mg/kg males was less than that of the vehicle control group and that of 20 mg/kg females. Heart weights of 20 mg/kg males were significantly greater than those of the vehicle controls. Collectively, the differences in the survival rates, mean body weight gains, and the increase in heart weights of males suggest that male mice may be more sensitive to acrylonitrile than female mice. Mild hematologic effects were observed in mice surviving to the end of the study; the hematologic changes were generally minimal and were more prevalent in females. Cyanide formation and the subsequent formation of cyanohemoglobin may have increased red blood cell turnover resulting in a minor decrease in erythrocytes. Acrylonitrile binding to hemoglobin may contribute to increased red blood cell turnover (Ahmed *et al.,* 1982). Similar hematologic effects were observed in mice treated with chemicals metabolized via epoxidation such as 1,3-butadiene (NTP, 1993).

Histopathologic lesions of the forestomach including inflammation, ulcer, and hyperplasia were observed in 40 mg/kg female mice in the 14-week study. No forestomach lesions were observed in male or female mice dosed with 60 mg/kg acrylonitrile, most likely because all but one female died on the first day of the study and there was not time for forestomach lesions to develop. No lesions were observed in male or female mice that received 20 mg/kg acrylonitrile or less for 14 weeks.

Doses for the 2-year study were selected on the basis of the results of the 14-week study. Due to the high mortality in the 40 and 60 mg/kg groups and increased incidences of forestomach lesions in 40 mg/kg females, doses selected for the 2-year carcinogenicity study were

0, 2.5, 10, or 20 mg/kg per day. Because acrylonitrile was carcinogenic in rats regardless of the route of administration (gavage, drinking water, or inhalation) and because there were similarities in the target organs in these studies (USEPA, 1983; WHO, 1983; ATSDR, 1990; IARC, 1999), gavage administration was selected as the exposure route in the current 2-year study.

In the current 2-year study, survival of 20 mg/kg male and female mice was significantly less than that of the vehicle control groups. Mean body weights of 20 mg/kg males and females were generally less than those of the vehicle controls throughout most of the study.

Dose-related increases in the incidences of forestomach neoplasms occurred in male and female mice. The incidences of forestomach squamous cell papilloma or carcinoma (combined) occurred with positive trends in males and females, and the incidences of these neoplasms in 10 and 20 mg/kg mice were significantly increased. Dose-related increases in the incidences of nonneoplastic forestomach lesions including focal epithelial hyperplasia were also observed; these increases were statistically significant in 20 mg/kg males and females.

Forestomach tumors were observed in rats that received acrylonitrile by gavage, inhalation, or in drinking water (USEPA, 1983; WHO, 1983; ATSDR, 1990; IARC, 1999). The mechanisms of acrylonitrile-induced forestomach tumors are not fully understood; however, an association between chemical-induced forestomach epithelial hyperplasia and carcinogenesis has been proposed (Ghanayem *et al.,* 1986). Proliferative epithelial forestomach lesions may be indicative of carcinogenicity and may constitute a continuum progressing to papilloma and then to carcinoma (Ghanayem *et al.,* 1994; Leininger *et al.,* 1999). However, while increased incidences of epithelial hyperplasia of the forestomach occurred in mice that received 20 mg/kg acrylonitrile in the current 2-year study, no such lesions were observed at lower doses that caused forestomach neoplasms. Further, forestomach hyperplasia was not observed at 20 mg/kg or less in the 14-week study. Therefore, it is possible that this mechanism may not be the only contributing factor in the pathogenesis of forestomach carcinogenesis in mice. High concentrations of acrylonitrile and/or its metabolites were detected in the stomach of rats treated intravenously or orally and may be related to carcinogenesis at this site (Burka *et al.,* 1994).

More recently, the effects of acrylonitrile on cell proliferation and programmed cell death were investigated in the forestomach (target of carcinogenicity), glandular stomach, and liver of male F344 rats dosed with acrylonitrile by gavage for 6 weeks (Ghanayem *et al.,* 1997). Immunohistochemical staining for the quantitation of bromodeoxyuridine incorporation into the DNA demonstrated that acrylonitrile caused a dose-dependent enhancement of mucosal cell proliferation in the forestomach in association with a parallel increase in programmed cell death. Acrylonitrile induced a net increase in mucosal cell proliferation in association with increased forestomach thickness. In contrast, acrylonitrile had no significant effect on cell proliferation in the liver or glandular stomach of treated rats. It was suggested that acrylonitrile caused disruption of the balance between cell proliferation and programmed cell death in favor of a net enhancement of epithelial cell proliferation in the forestomach, which may have contributed to its carcinogenicity.

Acrylonitrile also caused increases in the incidences of harderian gland neoplasms in male and female mice in the 2-year study. Increases in the incidences of harderian gland hyperplasia occurred in dosed males and females. Botts *et al.* (1999) suggested that primary hyperplasia is a precursor to the development of neoplastic lesions and that it may be a contributing factor in chemical-induced harderian gland carcinogenesis. In contrast to the present effects in mice, no increases in the incidences of harderian gland tumors were reported in rats treated with acrylonitrile (USEPA, 1983; WHO, 1983; ATSDR 1990; IARC, 1999). However, harderian gland tumors are uncommon in rats and are rarely observed in NTP studies. The harderian gland is a secretory gland located medial and posterior to the globe of the eye. In the current 2-year study, the observed large proliferative lesions of the harderian gland may explain the grossly observable compression and protrusion (proptosis) of the eye in dosed males and females. The eye lesions were invariably associated with the presence of harderian gland neoplasms, and they were consistent with ocular compression by these neoplasms; therefore, the increases in the incidences of eye lesions observed in the present study were considered secondary to acrylonitrile-induced harderian gland neoplasms.

Neoplasms of the ovary were also observed in dosed female mice at 2 years. The incidences of atrophy and cyst were significantly increased in 10 and 20 mg/kg females, and atrophy was characterized by partial to

complete lack of histologically evident follicular and corpus luteum development. Although the increased incidences of combined benign or malignant ovarian granulosa cell neoplasms in dosed female mice were not statistically significant, they may have been related to acrylonitrile administration. Ovarian neoplasms in control mice are rare, and the incidences of these neoplasms in the 10 and 20 mg/kg female groups from this study exceeded the NTP historical control ranges.

Out of over 400 NTP carcinogenicity studies, eight chemicals were identified that significantly increased ovarian neoplasms such as tubular adenomas, granulosa cell tumors, and mixed tumors in mice, but none have caused tumors in rats (Davis and Maronpot, 1996). Most of these chemicals are mutagenic, and most are associated with lung and/or mammary gland tumors. The relationship between ovarian and lung neoplasms is not clear. All eight ovarian carcinogens are also associated with ovarian damage and epithelial hyperplasia, and these changes may precede development of ovarian neoplasia. However, not all ovarian carcinogens are necessarily associated with decreasing oocyte (follicle) counts. Furthermore, not all chemicals that decrease follicle numbers induce ovarian tumors under NTP bioassay conditions.

Particularly in mice, due to decreases in estrogen production by the ovary from a variety of causes, there is a disruption of negative feedback control that results in excessive production of luteinizing hormone by the pituitary gland, leading to hyperplastic and neoplastic responses by the ovarian tissue (Capen, 2001). Factors such as senescence, genetic deletion of follicles, X-irradiation, drugs, and xenobiotic chemicals that destroy or greatly diminish the number of ovarian follicles or diminish sex steroid hormone by the ovary increase the risk of developing ovarian tubular adenoma and/or granulosa cell tumors.

In the 2-year study, acrylonitrile also may have caused increases in the incidences of lung neoplasms in female mice. The incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in 10 and 20 mg/kg females were greater than those in the vehicle control group; the incidence in the 10 mg/kg group was significantly increased. There was a slight increase in the incidence of hyperplasia of the alveolar epithelium in dosed females. The low incidence of alveolar/bronchiolar adenoma or carcinoma (combined) in 20 mg/kg females may have been due to the decreased survival in these groups. No increases in the incidences of lung neoplasms in dosed male mice were observed. In contrast to the present effect in mice, no increases in the incidences of lung neoplasms in acrylonitrile-treated rats have been observed in previous studies (USEPA, 1983; WHO, 1983; ATSDR, 1990; IARC, 1999).

As previously mentioned, the carcinogenicity of acrylonitrile has been investigated extensively in gavage, drinking water, and inhalation studies in rats. Generally, acrylonitrile caused an increase in the incidences of brain, Zymbal's gland, forestomach, and mammary gland tumors. In the current 2-year studies, acrylonitrile caused increases in the incidences of forestomach and harderian gland neoplasms in male and female mice and caused increases in the incidences of neoplasms of the ovary and lung in female mice. Therefore, it is clear that there are differences in the target organs of acrylonitrile carcinogenicity among rats and mice. The forestomach is the only common target organ of acrylonitrile carcinogenicity in both species. In comparison to earlier studies in rats, there is no evidence of central nervous system (e.g., brain) acrylonitrile carcinogenicity in mice. It is well established that spontaneous brain tumors are rare in mice, and mice have proven to be resistant to chemical-induced central nervous system tumors (Radovsky and Mahler, 1999). No explanation is currently available to explain the greater susceptibility of rats to brain tumors.

The pattern of acrylonitrile-induced carcinogenicity in rats and mice greatly resembles the pattern observed in animals treated with 1,3-butadiene, vinyl chloride, benzene, or ethylene oxide (Melnick and Huff, 1993). While brain neoplasms were observed in rats treated with 1,3-butadiene, ethylene oxide, vinyl chloride, or acrylonitrile, lung neoplasms were reported only in mice. Zymbal's gland neoplasms were reported in rats treated with 1,3-butadiene, vinyl chloride, or acrylonitrile and in rats and mice treated with benzene. Mice appeared more sensitive to harderian gland neoplasms and these neoplasms were observed in mice treated with 1,3-butadiene, benzene, or ethylene oxide. Ovarian neoplasms were also more prevalent in mice treated with 1,3-butadiene or benzene. Forestomach neoplasms were

observed in mice treated with 1,3-butadiene or benzene and in rats treated with benzene or vinyl chloride (Huff *et al.,* 1989; Melnick and Huff, 1993). All five chemicals are epoxides in nature (ethylene oxide) or are metabolized to mutagenic epoxide intermediates via cytochrome P450 2E1 (Ghanayem *et al.,* 2000). Whether this property is related to the carcinogenic activity of these chemicals remains to be established; however, bioactivation through epoxide formation may play a role (Ghanayem *et al.,* 2000).

Acrylonitrile CYP2E1-mediated metabolism was implicated in the mutagenicity and carcinogenicity of this chemical, and cyanoethylene oxide (epoxide intermediate) is considered by many as the ultimate mutagenic/carcinogenic metabolite. Significant species-dependent variations in the metabolism and disposition of acrylonitrile have been reported. The ratio of acrylonitrile epoxidation to direct GSH conjugation is greater in mice than in rats (Kedderis and Batra, 1993). Mouse hepatic microsomes metabolize acrylonitrile to cyanoethylene oxide at higher rates than those of rats and humans, both of which epoxidize acrylonitrile at approximately the same rate (Kedderis *et al.,* 1993b). Mice also excrete a significantly greater percentage of the administered dose as metabolites originating from the cyanoethylene oxide pathway than rats (Fennell *et al.,* 1991), and they excrete more urinary thiocyanates than rats (Gut *et al.,* 1975). It remains unclear whether species differences in the metabolism of acrylonitrile explain the differences in the target organs of acrylonitrile carcinogenicity in rats and mice.

Urinary thiocyanate and *N*-acetyl-S-(2-cyanoethyl)-L-cysteine were measured at various time points during the study as indicators of internal dose. While acrylonitrile metabolism to cyanide (and subsequently to thiocyanate) is considered a marker of a cytochrome P450-mediated epoxidation pathway, metabolism to *N*-acetyl-S-(2-cyanoethyl)-L-cysteine is considered a marker of direct conjugation of parent acrylonitrile with reduced glutathione (Figure 1). Generally, dose-related increases in urinary thiocyanate and *N*-acetyl-S-(2-cyanoethyl)-L-cysteine concentrations in the current study suggest that higher doses of acrylonitrile result in higher urinary metabolite concentrations (Figures 4 and 5). Further, excretion of each metabolite apparently increased at 12 and 18 months compared to 2 weeks and 3 months. The reasons for these consistent differences are unclear. However, these data did confirm that exposure of mice to acrylonitrile in the current study was accomplished as intended. Further, contrary to earlier reports (Roberts *et al.,* 1989, 1991; Kedderis *et al.,* 1993b; Gargas *et al.,* 1995), evidence for saturation of the epoxidation pathway was not revealed by these data.

Acrylonitrile mutagenicity varied greatly from one test system to another and between laboratories. In the present studies, acrylonitrile was mutagenic in *Salmonella typhimurium* strains TA100 and TA1535 with S9 metabolic activation and was negative in TA97 and TA98, with and without S9. In cultured Chinese hamster ovary cells, acrylonitrile induced sister chromatid exchanges with and without S9 activation. However, chromosomal aberrations in these cells were significantly increased only in the presence of S9. Acrylonitrile failed to cause sex-linked recessive lethal mutations or reciprocal translocations in *Drosophila melanogaster*. Mice treated with up to 40 mg/kg acrylonitrile for 14 weeks showed no increases in micronucleated erythrocytes in peripheral blood. There were enough acrylonitrile metabolites in urine from gavage or intraperitoneally dosed mice to cause mutagenicity when the urine was applied to the *Salmonella* (Lambotte-Vandepaer *et al.,* 1980, 1981, 1985).

Studies that described the binding of acrylonitrile with macromolecules were generally inconsistent. Acrylonitrile was shown to covalently bind with rat liver microsomal proteins in a time-dependent manner without metabolic activation (Peter and Bolt, 1981). Protein binding but not DNA binding of acrylonitrile apparently occurs via direct alkylation because it does not require NADPH (Guengerich *et al.,* 1981). However, in the presence of NADPH, DNA binding was detectable (Guengerich *et al.,* 1981). Cyanoethylene oxide also covalently bound to calf thymus DNA and rat microsomal proteins (Guengerich *et al.,* 1981). Intraperitoneal administration of cyanoethylene oxide resulted in binding to liver and brain proteins but not to DNA (Hogy and Guengerich, 1986). In another study, acrylonitrile failed to induce unscheduled DNA synthesis in rat liver and spermatocytes (Butterworth *et al,* 1992).

Acrylonitrile increased oxidative DNA damage in rat brain, which was evident by the presence of 8-hydroxy-2′-deoxyguanosine, products of lipid peroxidation, and reactive oxygen species (Jiang *et al.,* 1998). These effects were not observed in the liver of these animals. Additionally, *in vitro* incubation of acrylonitrile with a rat glial cell line or hepatocytes at 0.01 to 1.0 mM showed that 8-hydroxy-2′-deoxyguanosine levels and hydroxy radical formation increased in rat glial cells but not in rat hepatocytes (Kamendulis *et al.,* 1999a). Acrylonitrile caused a dose-dependent inhibition of gap junctional intercellular communication in a rat astrocyte cell line (Kamendulis *et al.,* 1999b), a property that is considered more characteristic of nongenotoxic carcinogens.

CONCLUSIONS

Under the conditions of this 2-year gavage study, there was *clear evidence of carcinogenic activity** of acrylonitrile in male and female $B6C3F_1$ mice based on increased incidences of forestomach and harderian gland neoplasms. Neoplasms of the ovary and lung in female mice may have been related to administration of acrylonitrile.

Nonneoplastic lesions of the forestomach and harderian gland in males and of the forestomach and ovary in females were associated with administration of acrylonitrile by gavage for 2 years.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 8. A summary of the Technical Reports Review Subcommittee comments and public discussion on this Technical Report appears on page 10.

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APPENDIX A
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR GAVAGE STUDY OF ACRYLONITRILE

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Table A1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Acrylonitrile[a]

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths		2		
Moribund	1	3	3	4
Natural deaths	11	3	8	32
Survivors				
Terminal sacrifice	38	42	39	14
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(50)	(49)	(50)	(48)
Squamous cell carcinoma, metastatic, stomach, forestomach				1 (2%)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Polyp adenomatous	1 (2%)	3 (6%)		
Intestine small, jejunum	(50)	(50)	(50)	(50)
Carcinoma	2 (4%)	1 (2%)		
Intestine small, ileum	(50)	(50)	(50)	(50)
Hemangioma				1 (2%)
Hemangiosarcoma				1 (2%)
Liver	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	1 (2%)	1 (2%)
Hemangiosarcoma	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Hemangiosarcoma, multiple	1 (2%)			1 (2%)
Hepatoblastoma		1 (2%)		
Hepatocellular carcinoma	11 (22%)	9 (18%)	13 (26%)	6 (12%)
Hepatocellular carcinoma, multiple	3 (6%)	1 (2%)	4 (8%)	
Hepatocellular adenoma	13 (26%)	21 (42%)	11 (22%)	10 (20%)
Hepatocellular adenoma, multiple	10 (20%)	11 (22%)	18 (36%)	4 (8%)
Histiocytic sarcoma				1 (2%)
Osteosarcoma, metastatic, bone		1 (2%)		
Squamous cell carcinoma, metastatic, stomach, forestomach			4 (8%)	4 (8%)
Mesentery	(8)	(6)	(8)	(3)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (17%)		
Hemangioma		1 (17%)		
Sarcoma	1 (13%)			
Squamous cell carcinoma, metastatic, stomach, forestomach			1 (13%)	
Fat, carcinoma, metastatic, harderian gland			1 (13%)	
Fat, squamous cell carcinoma, metastatic, stomach, forestomach			2 (25%)	2 (67%)
Pancreas	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Sarcoma, metastatic, mesentery	1 (2%)			
Squamous cell carcinoma, metastatic, stomach, forestomach			3 (6%)	2 (4%)
Salivary glands	(50)	(50)	(50)	(50)

Table A1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Alimentary System (continued)				
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell carcinoma			8 (16%)	9 (18%)
Squamous cell papilloma	3 (6%)	4 (8%)	17 (34%)	16 (32%)
Squamous cell papilloma, multiple			2 (4%)	9 (18%)
Stomach, glandular	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)	
Squamous cell carcinoma, metastatic, stomach, forestomach			3 (6%)	2 (4%)
Tongue				(2)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (50%)
Squamous cell carcinoma				1 (50%)
Tooth	(9)	(5)	(4)	(3)
Peridontal tissue, sarcoma				1 (33%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	1 (2%)	
Hemangioma				1 (2%)
Hemangiosarcoma			1 (2%)	
Schwannoma malignant	1 (2%)			
Pericardium, hepatocellular carcinoma, metastatic, liver	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)	1 (2%)
Squamous cell carcinoma, metastatic, stomach, forestomach			1 (2%)	
Subcapsular, adenoma	3 (6%)		3 (6%)	
Adrenal medulla	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)	1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	2 (4%)		2 (4%)	
Pituitary gland	(49)	(46)	(47)	(47)
Pars intermedia, adenoma	1 (2%)			
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma		1 (2%)	1 (2%)	
Follicular cell, carcinoma		1 (2%)		
General Body System				
Peritoneum				(1)
Squamous cell carcinoma, metastatic, stomach, forestomach				1 (100%)

Table A1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Genital System				
Coagulating gland	(1)		(2)	
Epididymis	(50)	(50)	(50)	(50)
Sarcoma, metastatic, mesentery	1 (2%)			
Preputial gland	(49)	(50)	(50)	(50)
Hemangioma			1 (2%)	
Prostate	(50)	(50)	(50)	(50)
Squamous cell carcinoma, metastatic, stomach, forestomach			1 (2%)	
Seminal vesicle	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)		
Carcinoma	1 (2%)			
Sarcoma, metastatic, mesentery	1 (2%)			
Testes	(50)	(50)	(50)	(50)
Interstitial cell, adenoma		1 (2%)	1 (2%)	
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma	2 (4%)			
Lymph node	(6)	(4)	(6)	(5)
Bronchial, squamous cell carcinoma, metastatic, stomach, forestomach				1 (20%)
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung	1 (17%)	1 (25%)	1 (17%)	1 (20%)
Mediastinal, carcinoma, metastatic, harderian gland			1 (17%)	
Mediastinal, hepatocellular carcinoma, metastatic, liver	1 (17%)			
Mediastinal, sarcoma, metastatic, mesentery	1 (17%)			
Mediastinal, squamous cell carcinoma, metastatic, stomach, forestomach			1 (17%)	
Pancreatic, squamous cell carcinoma, metastatic, stomach, forestomach			1 (17%)	1 (20%)
Renal, alveolar/bronchiolar carcinoma, metastatic, lung		1 (25%)		
Lymph node, mandibular	(49)	(47)	(47)	(47)
Lymph node, mesenteric	(48)	(48)	(49)	(48)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)	
Hemangioma	2 (4%)	1 (2%)		
Histiocytic sarcoma				1 (2%)
Squamous cell carcinoma, metastatic, stomach, forestomach				2 (4%)
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)		1 (2%)	
Histiocytic sarcoma				1 (2%)
Squamous cell carcinoma, metastatic, stomach, forestomach			1 (2%)	
Thymus	(47)	(47)	(44)	(46)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)	1 (2%)	1 (2%)	
Hepatocellular carcinoma, metastatic, liver	1 (2%)			

Table A1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Subcutaneous tissue, fibrosarcoma		1 (2%)		
Subcutaneous tissue, hemangiosarcoma	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma		1 (2%)		1 (2%)
Osteosarcoma, multiple		1 (2%)		
Femur, fibrosarcoma	1 (2%)			
Skeletal muscle	(1)		(2)	
Carcinoma, metastatic, stomach, forestomach			1 (50%)	
Sarcoma, metastatic, mesentery	1 (100%)			
Squamous cell carcinoma, metastatic, stomach, forestomach			1 (50%)	
Nervous System				
None				
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	10 (20%)	8 (16%)	4 (8%)	6 (12%)
Alveolar/bronchiolar adenoma, multiple		1 (2%)		2 (4%)
Alveolar/bronchiolar carcinoma	4 (8%)	2 (4%)	4 (8%)	2 (4%)
Alveolar/bronchiolar carcinoma, multiple			1 (2%)	
Alveolar/bronchiolar carcinoma, metastatic, lung	2 (4%)	2 (4%)	2 (4%)	
Carcinoma, metastatic, harderian gland			3 (6%)	1 (2%)
Carcinoma, metastatic, thyroid gland		1 (2%)		
Hepatocellular carcinoma, metastatic, liver	5 (10%)	3 (6%)	4 (8%)	2 (4%)
Histiocytic sarcoma				1 (2%)
Osteosarcoma, metastatic, bone		1 (2%)		
Squamous cell carcinoma, metastatic, stomach, forestomach			4 (8%)	1 (2%)
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	1 (2%)	1 (2%)
Mediastinum, hepatocellular carcinoma, metastatic, liver	1 (2%)			
Mediastinum, osteosarcoma, metastatic, bone		1 (2%)		
Nose	(50)	(50)	(50)	(50)
Carcinoma, metastatic, harderian gland			1 (2%)	
Special Senses System				
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	5 (10%)	16 (32%)	15 (30%)	21 (42%)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Carcinoma	1 (2%)	1 (2%)	4 (8%)	3 (6%)
Bilateral, adenoma			9 (18%)	6 (12%)

Table A1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		2 (4%)		1 (2%)
Squamous cell carcinoma, metastatic, stomach, forestomach			1 (2%)	1 (2%)
Capsule, carcinoma, metastatic, harderian gland			1 (2%)	
Capsule, hepatocellular carcinoma, metastatic, liver	1 (2%)			
Renal tubule, adenoma	1 (2%)	1 (2%)	1 (2%)	
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma				1 (2%)
Lymphoma malignant	3 (6%)	3 (6%)	1 (2%)	3 (6%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	45	43	49	41
Total primary neoplasms	85	92	125	106
Total animals with benign neoplasms	34	39	45	39
Total benign neoplasms	51	69	86	76
Total animals with malignant neoplasms	29	18	30	24
Total malignant neoplasms	34	23	39	30
Total animals with metastatic neoplasms	8	7	13	9
Total metastatic neoplasms	19	19	47	30

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

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: Vehicle Control

Number of Days on Study	464	528	582	607	617	633	636	664	712	714	723	723	730	730	730	730	730	731	731	731	731	731	731	731	731
Carcass ID Number	043	003	020	017	048	012	042	049	028	029	004	019	007	009	021	024	031	001	006	010	015	016	018	022	023
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous								X																	
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																				X					
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																X									
Hemangiosarcoma, multiple																									
Hepatocellular carcinoma		X			X						X			X					X						
Hepatocellular carcinoma, multiple	X							X																	
Hepatocellular adenoma				X	X									X	X					X		X			
Hepatocellular adenoma, multiple							X						X			X	X	X							X
Mesentery								+		+									+			+			
Sarcoma										X															
Oral mucosa										+	+														
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, mesentery										X															
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma	X																X				X				
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth										+												+			+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant																									
Pericardium, hepatocellular carcinoma, metastatic, liver								X																	
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcapsular, adenoma																			X				X		
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma														X											
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	M	+	M
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Pars intermedia, adenoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: Vehicle Control

Number of Days on Study	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	026	032	034	036	038	039	044	046	047	050	002	005	008	011	013	014	025	027	030	033	035	037	040	041	045	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp adenomatous																										1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma														X												2
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Hemangiosarcoma, multiple															X											1
Hepatocellular carcinoma		X			X	X									X	X	X									11
Hepatocellular carcinoma, multiple	X																									3
Hepatocellular adenoma	X			X					X			X	X								X	X				13
Hepatocellular adenoma, multiple					X						X						X			X						10
Mesentery	+			+								+	+													8
Sarcoma																										1
Oral mucosa																										2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, metastatic, mesentery																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																										3
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth			+	+								+		+								+			+	9
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma malignant																									X	1
Pericardium, hepatocellular carcinoma, metastatic, liver																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcapsular, adenoma																				X						3
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																	X									2
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	42
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars intermedia, adenoma	X																									1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: Vehicle Control

Number of Days on Study	464	528	582	607	617	633	636	664	712	714	723	723	730	730	730	730	730	731	731	731	731	731	731	731	731
Carcass ID Number	043	003	020	017	048	012	042	049	028	029	004	019	007	009	021	024	031	001	006	010	015	016	018	022	023
General Body System																									
None																									
Genital System																									
Coagulating gland																						+			
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, mesentery										X															
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Sarcoma, metastatic, mesentery										X															
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma			X																						
Lymph node						+		+		+		+										+			
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung						X																			
Mediastinal, hepatocellular carcinoma, metastatic, liver								X																	
Mediastinal, sarcoma, metastatic, mesentery										X															
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	M	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma											X				X										
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma			X																						
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung						X																			
Hepatocellular carcinoma, metastatic, liver								X																	
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, hemangiosarcoma			X																						
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Femur, fibrosarcoma																									
Skeletal muscle										+															
Sarcoma, metastatic, mesentery										X															
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve																									

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: Vehicle Control

Number of Days on Study	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	026	032	034	036	038	039	044	046	047	050	002	005	008	011	013	014	025	027	030	033	035	037	040	041	045	Total Tissues/ Tumors
General Body System																										
None																										
Genital System																										
Coagulating gland																										1
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, metastatic, mesentery																										1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																				X						1
Sarcoma, metastatic, mesentery																										1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma															X											2
Lymph node																					+					6
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Mediastinal, hepatocellular carcinoma, metastatic, liver																										1
Mediastinal, sarcoma, metastatic, mesentery																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hemangioma																										2
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	47
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Hepatocellular carcinoma, metastatic, liver																										1
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	+	M	M	M	M	M	M	M	M	3
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, hemangiosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Femur, fibrosarcoma											X															1
Skeletal muscle																										1
Sarcoma, metastatic, mesentery																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve														+												1

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: Vehicle Control

Number of Days on Study	464	528	582	607	617	633	636	664	712	714	723	723	730	730	730	730	730	731	731	731	731	731	731	731	731
Carcass ID Number	043	003	020	017	048	012	042	049	028	029	004	019	007	009	021	024	031	001	006	010	015	016	018	022	023
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma										X									X		X				X
Alveolar/bronchiolar carcinoma					X	X	X		X																
Alveolar/bronchiolar carcinoma, metastatic, lung						X			X																
Hepatocellular carcinoma, metastatic, liver	X							X			X														
Mediastinum, hepatocellular carcinoma, metastatic, liver								X																	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																		X						X	
Carcinoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Capsule, hepatocellular carcinoma, metastatic, liver											X														
Renal tubule, adenoma																								X	
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant												X										X			

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: Vehicle Control

Number of Days on Study	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	026	032	034	036	038	039	044	046	047	050	002	005	008	011	013	014	025	027	030	033	035	037	040	041	045	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma	X		X						X							X	X						X			10
Alveolar/bronchiolar carcinoma																										4
Alveolar/bronchiolar carcinoma, metastatic, lung																										2
Hepatocellular carcinoma, metastatic, liver		X				X																				5
Mediastinum, hepatocellular carcinoma, metastatic, liver																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma												X	X			X										5
Carcinoma							X																			1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Capsule, hepatocellular carcinoma, metastatic, liver																										1
Renal tubule, adenoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant																					X					3

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: 2.5 mg/kg

Number of Days on Study	003	004	518	535	637	689	689	714	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731
Carcass ID Number	063	059	083	091	095	066	082	053	069	072	073	075	078	086	087	093	099	051	054	056	058	060	061	064	067
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous																					X		X		
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung				X																					
Hemangiosarcoma																									X
Hepatoblastoma							X																		
Hepatocellular carcinoma				X	X	X	X									X									
Hepatocellular carcinoma, multiple																									
Hepatocellular adenoma									X						X	X	X	X						X	X
Hepatocellular adenoma, multiple												X							X		X	X	X		
Osteosarcoma, metastatic, bone								X																	
Mesentery						+														+					
Alveolar/bronchiolar carcinoma, metastatic, lung						X																			
Hemangioma																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma														X											X
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth										+										+		+			
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung				X																					
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	M	+	M	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma								X																	
Follicular cell, carcinoma			X																						
General Body System																									
None																									

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: 2.5 mg/kg

Number of Days on Study	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	068	070	071	080	081	084	085	092	098	052	055	057	062	065	074	076	077	079	088	089	090	094	096	097	010	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp adenomatous					X																					3
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma					X																					1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Hemangiosarcoma																										1
Hepatoblastoma																										1
Hepatocellular carcinoma					X	X	X			X																9
Hepatocellular carcinoma, multiple				X																						1
Hepatocellular adenoma					X	X	X		X	X	X	X	X	X	X			X		X	X	X				21
Hepatocellular adenoma, multiple				X				X								X			X				X	X		11
Osteosarcoma, metastatic, bone																										1
Mesentery								+		+								+						+		6
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Hemangioma																		X								1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma												X	X													4
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth												+						+								5
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	44
Pituitary gland	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	46
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																										1
Follicular cell, carcinoma																										1
General Body System																										
None																										

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: 2.5 mg/kg

Number of Days on Study	003	004	518	535	637	689	689	714	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731
Carcass ID Number	063	059	083	091	095	066	082	053	069	072	073	075	078	086	087	093	099	051	054	056	058	060	061	064	067	
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Alveolar/bronchiolar carcinoma, metastatic, lung						X																				
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Interstitial cell, adenoma																										
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lymph node						+									+					+						
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung						X																				
Renal, alveolar/bronchiolar carcinoma, metastatic, lung						X																				
Lymph node, mandibular	+	+	+	+	+	M	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lymph node, mesenteric	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Hemangioma																										
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Thymus	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	
Alveolar/bronchiolar carcinoma, metastatic, lung						X																				
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Subcutaneous tissue, fibrosarcoma																										
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Osteosarcoma			X																							
Osteosarcoma, multiple								X																		
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Alveolar/bronchiolar adenoma													X						X					X		
Alveolar/bronchiolar adenoma, multiple																										
Alveolar/bronchiolar carcinoma				X		X																				
Alveolar/bronchiolar carcinoma, metastatic, lung				X		X																				
Carcinoma, metastatic, thyroid gland			X																							
Hepatocellular carcinoma, metastatic, liver					X																					
Osteosarcoma, metastatic, bone								X																		
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung						X																				
Mediastinum, osteosarcoma, metastatic, bone								X																		
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: 2.5 mg/kg

Number of Days on Study	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	068	070	071	080	081	084	085	092	098	052	055	057	062	065	074	076	077	079	088	089	090	094	096	097	100	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma																						X				1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node											+															4
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Renal, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hemangioma										X																1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma																				X						1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																										1
Osteosarcoma, multiple																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma			X		X					X			X								X					8
Alveolar/bronchiolar adenoma, multiple												X														1
Alveolar/bronchiolar carcinoma																										2
Alveolar/bronchiolar carcinoma, metastatic, lung																										2
Carcinoma, metastatic, thyroid gland																										1
Hepatocellular carcinoma, metastatic, liver				X			X																			3
Osteosarcoma, metastatic, bone																										1
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Mediastinum, osteosarcoma, metastatic, bone																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: 2.5 mg/kg

Number of Days on Study	003	004	518	535	637	689	689	714	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731
Carcass ID Number	063	059	083	091	095	066	082	053	069	072	073	075	078	086	087	093	099	051	054	056	058	060	061	064	067
Special Senses System																									
Eye																									
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma			X			X	X					X						X	X		X		X		
Carcinoma																									
Zymbal's gland																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung				X		X																			
Renal tubule, adenoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant																				X					

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: 2.5 mg/kg

Number of Days on Study	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	068	070	071	080	081	084	085	092	098	052	055	057	062	065	074	076	077	079	088	089	090	094	096	097	010	Total Tissues/ Tumors
Special Senses System																										
Eye				+																						1
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma				X	X	X	X		X		X		X										X			16
Carcinoma																							X			1
Zymbal's gland													+													1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										2
Renal tubule, adenoma																								X		1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant											X						X									3

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: 10 mg/kg

Number of Days on Study	211	497	512	515	563	610	626	685	702	709	714	730	730	730	730	730	730	730	730	730	730	730	730	731	731
Carcass ID Number	122	147	124	103	149	112	146	120	132	111	104	101	115	117	129	131	133	138	140	143	145	148	150	107	109
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung					X																				
Hemangiosarcoma																			X						
Hepatocellular carcinoma					X	X			X	X	X		X				X						X		X
Hepatocellular carcinoma, multiple				X										X											
Hepatocellular adenoma					X						X					X					X			X	
Hepatocellular adenoma, multiple						X						X		X	X				X			X			X
Squamous cell carcinoma, metastatic, stomach, forestomach				X			X	X	X																
Mesentery				+				+	+													+			
Squamous cell carcinoma, metastatic, stomach, forestomach				X																					
Fat, carcinoma, metastatic, harderian gland																									
Fat, squamous cell carcinoma, metastatic, stomach, forestomach								X	X																
Oral mucosa																	+								
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, stomach, forestomach				X				X	X																
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma				X			X	X	X									X							
Squamous cell papilloma										X					X	X			X	X		X		X	X
Squamous cell papilloma, multiple																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung					X																				
Squamous cell carcinoma, metastatic, stomach, forestomach							X	X	X																
Tooth																	+					+	+		
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung					X																				
Hemangiosarcoma																									X

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: 10 mg/kg

Number of Days on Study	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	110	116	118	119	121	125	126	130	135	144	102	105	106	108	113	114	123	127	128	134	136	137	139	141	142	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Hemangiosarcoma															X											2
Hepatocellular carcinoma						X				X	X														X	13
Hepatocellular carcinoma, multiple													X				X									4
Hepatocellular adenoma		X						X	X				X	X					X							11
Hepatocellular adenoma, multiple	X			X	X	X				X	X	X			X	X		X						X		18
Squamous cell carcinoma, metastatic, stomach, forestomach																										4
Mesentery										+	+		+					+								8
Squamous cell carcinoma, metastatic, stomach, forestomach																										1
Fat, carcinoma, metastatic, harderian gland											X															1
Fat, squamous cell carcinoma, metastatic, stomach, forestomach																										2
Oral mucosa						+								+				+								4
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma, metastatic, stomach, forestomach																										3
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma						X			X	X																8
Squamous cell papilloma		X			X	X	X	X							X					X			X		X	17
Squamous cell papilloma, multiple				X									X													2
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Squamous cell carcinoma, metastatic, stomach, forestomach																										3
Tooth								+																		4
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Hemangiosarcoma																										1

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: 10 mg/kg

Number of Days on Study	211	497	512	515	563	610	626	685	702	709	714	730	730	730	730	730	730	730	730	730	730	730	730	731	731
Carcass ID Number	122	147	124	103	149	112	146	120	132	111	104	101	115	117	129	131	133	138	140	143	145	148	150	107	109
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																X									
Alveolar/bronchiolar carcinoma, metastatic, lung					X																				
Squamous cell carcinoma, metastatic, stomach, forestomach									X																
Subcapsular, adenoma		X																							
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung					X																				
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma							X	X																	
Parathyroid gland	+	+	M	M	M	+	M	+	+	+	+	M	+	+	+	M	+	M	M	+	+	+	M	+	M
Pituitary gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																			X						
General Body System																									
None																									
Genital System																									
Coagulating gland																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, stomach, forestomach								X																	
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma																									

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: 10 mg/kg

Number of Days on Study	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	110	116	118	119	121	125	126	130	135	144	102	105	106	108	113	114	123	127	128	134	136	137	139	141	142	Total Tissues/ Tumors
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Squamous cell carcinoma, metastatic, stomach, forestomach																										1
Subcapsular, adenoma							X								X											3
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										2
Parathyroid gland	M	M	+	+	+	+	+	M	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	34
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	47
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																										1
General Body System																										
None																										
Genital System																										
Coagulating gland	+														+											2
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma											X															1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma, metastatic, stomach, forestomach																										1
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma																		X								1

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: 10 mg/kg

Number of Days on Study	211	497	512	515	563	610	626	685	702	709	714	730	730	730	730	730	730	730	730	730	730	730	730	731	731
Carcass ID Number	122	147	124	103	149	112	146	120	132	111	104	101	115	117	129	131	133	138	140	143	145	148	150	107	109
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node					+	+		+	+																
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung					X																				
Mediastinal, carcinoma, metastatic, harderian gland																									
Mediastinal, squamous cell carcinoma, metastatic, stomach, forestomach								X																	
Pancreatic, squamous cell carcinoma, metastatic, stomach, forestomach									X																
Lymph node, mandibular	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung					X																				
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma													X												
Squamous cell carcinoma, metastatic, stomach, forestomach				X																					
Thymus	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	M	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung					X																				
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle				+					+																
Carcinoma, metastatic, stomach, forestomach				X																					
Squamous cell carcinoma, metastatic, stomach, forestomach									X																
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+																								
Spinal cord	+																								

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: 10 mg/kg

Number of Days on Study	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	110	116	118	119	121	125	126	130	135	144	102	105	106	108	113	114	123	127	128	134	136	137	139	141	142	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node											+										+					6
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Mediastinal, carcinoma, metastatic, harderian gland											X															1
Mediastinal, squamous cell carcinoma, metastatic, stomach, forestomach																										1
Pancreatic, squamous cell carcinoma, metastatic, stomach, forestomach																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	49
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Squamous cell carcinoma, metastatic, stomach, forestomach																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	M	+	44
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										2
Carcinoma, metastatic, stomach, forestomach																										1
Squamous cell carcinoma, metastatic, stomach, forestomach																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord																										1

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: 10 mg/kg

Number of Days on Study	211	497	512	515	563	610	626	685	702	709	714	730	730	730	730	730	730	730	730	730	730	730	730	731	731
Carcass ID Number	122	147	124	103	149	112	146	120	132	111	104	101	115	117	129	131	133	138	140	143	145	148	150	107	109
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																								X	
Alveolar/bronchiolar carcinoma					X																				
Alveolar/bronchiolar carcinoma, multiple													X												
Alveolar/bronchiolar carcinoma, metastatic, lung					X																				
Carcinoma, metastatic, harderian gland		X																							
Hepatocellular carcinoma, metastatic, liver						X																			
Squamous cell carcinoma, metastatic, stomach, forestomach				X			X	X	X																
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung					X																				
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, harderian gland		X																							
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye		+			+																				
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma		X	X				X				X	X						X	X						
Carcinoma		X																						X	
Bilateral, adenoma				X	X			X	X													X			X
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, stomach, forestomach									X																
Capsule, carcinoma, metastatic, harderian gland																									
Renal tubule, adenoma															X										
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant																									

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: 10 mg/kg

Number of Days on Study	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	110	116	118	119	121	125	126	130	135	144	102	105	106	108	113	114	123	127	128	134	136	137	139	141	142	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma			X	X															X							4
Alveolar/bronchiolar carcinoma				X				X												X						4
Alveolar/bronchiolar carcinoma, multiple																										1
Alveolar/bronchiolar carcinoma, metastatic, lung								X																		2
Carcinoma, metastatic, harderian gland											X			X												3
Hepatocellular carcinoma, metastatic, liver						X											X								X	4
Squamous cell carcinoma, metastatic, stomach, forestomach																										4
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, harderian gland																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye												+		+				+				+				6
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma	X	X			X		X			X		X							X						X	15
Carcinoma											X			X												4
Bilateral, adenoma																		X			X	X				9
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma, metastatic, stomach, forestomach																										1
Capsule, carcinoma, metastatic, harderian gland											X															1
Renal tubule, adenoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant																					X					1

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: 20 mg/kg

Number of Days on Study	113	113	114	211	239	274	283	337	410	477	492	494	502	505	520	525	540	547	553	554	554	554	569	588	603
Carcass ID Number	196	200	189	176	166	190	169	187	171	170	177	199	191	185	178	197	180	174	179	152	161	167	195	153	158
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, stomach, forestomach																									
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																									
Hemangiosarcoma																									
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung																									
Hemangiosarcoma																									
Hemangiosarcoma, multiple																									
Hepatocellular carcinoma																									
Hepatocellular adenoma														X	X					X					
Hepatocellular adenoma, multiple																X									
Histiocytic sarcoma																								X	
Squamous cell carcinoma, metastatic, stomach, forestomach																		X							
Mesentery																+									
Fat, squamous cell carcinoma, metastatic, stomach, forestomach																X									
Oral mucosa																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung																									
Squamous cell carcinoma, metastatic, stomach, forestomach																		X							
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																X	X	X							
Squamous cell papilloma									X				X	X					X			X	X		
Squamous cell papilloma, multiple											X													X	
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, stomach, forestomach																		X							
Tongue												+													
Alveolar/bronchiolar carcinoma, metastatic, lung																									
Squamous cell carcinoma												X													
Tooth																								+	
Peridontal tissue, sarcoma																								X	
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																									

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: 20 mg/kg

Number of Days on Study	642	656	673	679	695	709	715	716	724	726	728	730	731	731	731	731	731	731	732	732	732	732	732	732	732	732	
Carcass ID Number	175	181	151	173	160	182	183	156	154	184	155	168	159	162	164	165	192	194	157	163	172	186	188	193	198		Total Tissues/ Tumors
Alimentary System																											
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+		48
Squamous cell carcinoma, metastatic, stomach, forestomach		X																									1
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Hemangioma																	X										1
Hemangiosarcoma																									X		1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Alveolar/bronchiolar carcinoma, metastatic, lung	X																										1
Hemangiosarcoma																			X								1
Hemangiosarcoma, multiple																						X					1
Hepatocellular carcinoma							X	X	X							X			X			X					6
Hepatocellular adenoma						X	X						X			X		X		X	X						10
Hepatocellular adenoma, multiple									X		X						X										4
Histiocytic sarcoma																											1
Squamous cell carcinoma, metastatic, stomach, forestomach		X	X		X																						4
Mesentery			+			+																					3
Fat, squamous cell carcinoma, metastatic, stomach, forestomach			X																								2
Oral mucosa								+																			1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Alveolar/bronchiolar carcinoma, metastatic, lung	X																										1
Squamous cell carcinoma, metastatic, stomach, forestomach		X																									2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Squamous cell carcinoma		X	X		X	X				X	X																9
Squamous cell papilloma	X					X	X		X	X		X	X					X	X						X		16
Squamous cell papilloma, multiple														X	X	X	X				X		X	X			9
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Squamous cell carcinoma, metastatic, stomach, forestomach					X																						2
Tongue	+																										2
Alveolar/bronchiolar carcinoma, metastatic, lung	X																										1
Squamous cell carcinoma																											1
Tooth								+												+							3
Peridontal tissue, sarcoma																											1
Cardiovascular System																											
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Hemangioma									X																		1

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: 20 mg/kg

Number of Days on Study	113	113	114	211	239	274	283	337	410	477	492	494	502	505	520	525	540	547	553	554	554	554	569	588	603
Carcass ID Number	196	200	189	176	166	190	169	187	171	170	177	199	191	185	178	197	180	174	179	152	161	167	195	153	158
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung																										
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung																										
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	M	+	+	M	+
Pituitary gland	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																										
Peritoneum																										
Squamous cell carcinoma, metastatic, stomach, forestomach																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node													+					+							
Bronchial, squamous cell carcinoma, metastatic, stomach, forestomach																									
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung																									
Pancreatic, squamous cell carcinoma, metastatic, stomach, forestomach																		X							
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	M	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Histiocytic sarcoma																								X	
Squamous cell carcinoma, metastatic, stomach, forestomach																X									
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																								X	
Thymus	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma																									

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: 20 mg/kg

Number of Days on Study	642	656	673	679	695	709	715	716	724	726	728	730	731	731	731	731	731	732	732	732	732	732	732	732	732		
Carcass ID Number	175	181	151	173	160	182	183	156	154	184	155	168	159	162	164	165	192	194	157	163	172	186	188	193	198		Total Tissues/ Tumors
Endocrine System																											
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Alveolar/bronchiolar carcinoma, metastatic, lung	X																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Alveolar/bronchiolar carcinoma, metastatic, lung	X																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	M	+	M	+	+	+	M	+	+	+	+	+	+	+	+		41
Pituitary gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
General Body System																											
Peritoneum			+																								1
Squamous cell carcinoma, metastatic, stomach, forestomach			X																								1
Genital System																											
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Hematopoietic System																											
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Lymph node	+	+		+																							5
Bronchial, squamous cell carcinoma, metastatic, stomach, forestomach		X																									1
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung	X																										1
Pancreatic, squamous cell carcinoma, metastatic, stomach, forestomach																											1
Lymph node, mandibular	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Lymph node, mesenteric	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Histiocytic sarcoma																											1
Squamous cell carcinoma, metastatic, stomach, forestomach		X																									2
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Histiocytic sarcoma																											1
Thymus	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+		46
Integumentary System																											
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M		
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Musculoskeletal System																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Osteosarcoma																								X			1

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: 20 mg/kg

Number of Days on Study	113	113	114	211	239	274	283	337	410	477	492	494	502	505	520	525	540	547	553	554	554	554	569	588	603
Carcass ID Number	196	200	189	176	166	190	169	187	171	170	177	199	191	185	178	197	180	174	179	152	161	167	195	153	158
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																										
Alveolar/bronchiolar adenoma, multiple																										
Alveolar/bronchiolar carcinoma																										
Carcinoma, metastatic, harderian gland																						X				
Hepatocellular carcinoma, metastatic, liver																										
Histiocytic sarcoma																								X		
Squamous cell carcinoma, metastatic, stomach, forestomach																										
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																										
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Special Senses System																										
Eye																						+				
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Adenoma										X		X				X	X		X						X	
Alveolar/bronchiolar carcinoma, metastatic, lung																										
Carcinoma																						X				
Bilateral, adenoma														X										X		
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Alveolar/bronchiolar carcinoma, metastatic, lung																										
Squamous cell carcinoma, metastatic, stomach, forestomach																										
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Histiocytic sarcoma																								X		
Lymphoma malignant													X													

Table A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Acrylonitrile: 20 mg/kg

Number of Days on Study	642	656	673	679	695	709	715	716	724	726	728	730	731	731	731	731	731	731	732	732	732	732	732	732	732	732	
Carcass ID Number	175	181	151	173	160	182	183	156	154	184	155	168	159	162	164	165	192	194	157	163	172	186	188	193	198		Total Tissues/ Tumors
Nervous System																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Alveolar/bronchiolar adenoma					X	X				X								X					X		X		6
Alveolar/bronchiolar adenoma, multiple	X																X										2
Alveolar/bronchiolar carcinoma	X																					X					2
Carcinoma, metastatic, harderian gland																											1
Hepatocellular carcinoma, metastatic, liver								X	X																		2
Histiocytic sarcoma																											1
Squamous cell carcinoma, metastatic, stomach, forestomach		X																									1
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung	X																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Special Senses System																											
Eye							+																		+		3
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Adenoma	X				X	X		X			X	X		X	X	X		X	X	X		X	X		X		21
Alveolar/bronchiolar carcinoma, metastatic, lung	X																										1
Carcinoma							X			X																	3
Bilateral, adenoma			X	X													X							X			6
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Alveolar/bronchiolar carcinoma, metastatic, lung	X																										1
Squamous cell carcinoma, metastatic, stomach, forestomach		X																									1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Histiocytic sarcoma																											1
Lymphoma malignant				X										X													3

Table A3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Adrenal Cortex: Adenoma				
Overall rate[a]	3/50 (6%)	0/50 (0%)	4/50 (8%)	0/50 (0%)
Adjusted rate[b]	6.5%	0.0%	8.7%	0.0%
Terminal rate[c]	3/38 (8%)	0/42 (0%)	3/39 (8%)	0/14 (0%)
First incidence (days)	730 (T)	—[e]	497	—
Poly-3 test[d]	P=0.512N	P=0.119N	P=0.497	P=0.212N
Harderian Gland: Adenoma				
Overall rate	5/50 (10%)	16/50 (32%)	24/50 (48%)	27/50 (54%)
Adjusted rate	10.8%	34.1%	49.5%	74.4%
Terminal rate	5/38 (13%)	13/42 (31%)	16/39 (41%)	12/14 (86%)
First incidence (days)	730 (T)	518	497	477
Poly-3 test	P<0.001	P=0.006	P<0.001	P<0.001
Harderian Gland: Carcinoma				
Overall rate	1/50 (2%)	1/50 (2%)	4/50 (8%)	3/50 (6%)
Adjusted rate	2.2%	2.2%	8.7%	9.6%
Terminal rate	1/38 (3%)	1/42 (2%)	3/39 (8%)	0/14 (0%)
First incidence (days)	730 (T)	730 (T)	497	554
Poly-3 test	P=0.053	P=0.760	P=0.177	P=0.184
Harderian Gland: Adenoma or Carcinoma				
Overall rate	6/50 (12%)	16/50 (32%)	27/50 (54%)	30/50 (60%)
Adjusted rate	13.0%	34.1%	55.6%	81.3%
Terminal rate	6/38 (16%)	13/42 (31%)	19/39 (49%)	12/14 (86%)
First incidence (days)	730 (T)	518	497	477
Poly-3 test	P<0.001	P=0.014	P<0.001	P<0.001
Small Intestine (Duodenum): Adenomatous Polyp				
Overall rate	1/50 (2%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	2.2%	6.5%	0.0%	0.0%
Terminal rate	0/38 (0%)	3/42 (7%)	0/39 (0%)	0/14 (0%)
First incidence (days)	664	730 (T)	—	—
Poly-3 test	P=0.135N	P=0.302	P=0.505N	P=0.581N
Liver: Hepatocellular Adenoma				
Overall rate	23/50 (46%)	32/50 (64%)	29/50 (58%)	14/50 (28%)
Adjusted rate	48.6%	69.5%	62.6%	42.2%
Terminal rate	20/38 (53%)	32/42 (76%)	26/39 (67%)	6/14 (43%)
First incidence (days)	607	730 (T)	563	505
Poly-3 test	P=0.222N	P=0.029	P=0.119	P=0.370N
Liver: Hepatocellular Carcinoma				
Overall rate	14/50 (28%)	10/50 (20%)	17/50 (34%)	6/50 (12%)
Adjusted rate	29.0%	21.1%	36.1%	19.6%
Terminal rate	9/38 (24%)	6/42 (14%)	11/39 (28%)	3/14 (21%)
First incidence (days)	464	535	515	715
Poly-3 test	P=0.541	P=0.258N	P=0.304	P=0.259N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	32/50 (64%)	36/50 (72%)[f]	37/50 (74%)	17/50 (34%)
Adjusted rate	65.3%	76.1%	78.5%	51.2%
Terminal rate	25/38 (66%)	32/42 (76%)	31/39 (80%)	8/14 (57%)
First incidence (days)	464	535	515	505
Poly-3 test	P=0.157N	P=0.172	P=0.109	P=0.145N

Table A3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	10/50 (20%)	9/50 (18%)	4/50 (8%)	8/50 (16%)
Adjusted rate	21.6%	19.6%	8.8%	25.8%
Terminal rate	9/38 (24%)	9/42 (21%)	4/39 (10%)	4/14 (29%)
First incidence (days)	714	730 (T)	730 (T)	642
Poly-3 test	P=0.469N	P=0.505N	P=0.078N	P=0.443
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	4/50 (8%)	2/50 (4%)	5/50 (10%)	2/50 (4%)
Adjusted rate	8.5%	4.3%	10.9%	6.5%
Terminal rate	0/38 (0%)	0/42 (0%)	4/39 (10%)	1/14 (7%)
First incidence (days)	617	535	563	642
Poly-3 test	P=0.461	P=0.343N	P=0.479	P=0.544N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	14/50 (28%)	11/50 (22%)	8/50 (16%)	9/50 (18%)
Adjusted rate	29.5%	23.5%	17.5%	29.0%
Terminal rate	9/38 (24%)	9/42 (21%)	7/39 (18%)	5/14 (36%)
First incidence (days)	617	535	563	642
Poly-3 test	P=0.399N	P=0.335N	P=0.129N	P=0.578N
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	3/50 (6%)	4/50 (8%)	19/50 (38%)	25/50 (50%)
Adjusted rate	6.4%	8.7%	41.9%	69.5%
Terminal rate	2/38 (5%)	4/42 (10%)	18/39 (46%)	12/14 (86%)
First incidence (days)	464	730 (T)	709	410
Poly-3 test	P<0.001	P=0.489	P<0.001	P<0.001
Stomach (Forestomach): Squamous Cell Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	8/50 (16%)	9/50 (18%)
Adjusted rate	0.0%	0.0%	17.2%	27.3%
Terminal rate	0/38 (0%)	0/42 (0%)	4/39 (10%)	0/14 (0%)
First incidence (days)	—	—	515	525
Poly-3 test	P<0.001	—[g]	P=0.004	P<0.001
Stomach (Forestomach): Squamous Cell Papilloma or Carcinoma				
Overall rate	3/50 (6%)	4/50 (8%)	26/50 (52%)	32/50 (64%)
Adjusted rate	6.4%	8.7%	55.7%	83.3%
Terminal rate	2/38 (5%)	4/42 (10%)	21/39 (54%)	12/14 (86%)
First incidence (days)	464	730 (T)	515	410
Poly-3 test	P<0.001	P=0.489	P<0.001	P<0.001
All Organs: Hemangiosarcoma				
Overall rate	3/50 (6%)	1/50 (2%)	4/50 (8%)	3/50 (6%)
Adjusted rate	6.4%	2.2%	8.8%	9.8%
Terminal rate	2/38 (5%)	1/42 (2%)	4/39 (10%)	3/14 (21%)
First incidence (days)	582	730 (T)	730 (T)	730 (T)
Poly-3 test	P=0.193	P=0.310N	P=0.483	P=0.457
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	5/50 (10%)	3/50 (6%)	5/50 (10%)	5/50 (10%)
Adjusted rate	10.7%	6.5%	11.0%	16.4%
Terminal rate	3/38 (8%)	3/42 (7%)	5/39 (13%)	4/14 (29%)
First incidence (days)	582	730 (T)	730 (T)	724
Poly-3 test	P=0.211	P=0.365N	P=0.610	P=0.358

Table A3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
All Organs: Malignant Lymphoma				
Overall rate	3/50 (6%)	3/50 (6%)	1/50 (2%)	3/50 (6%)
Adjusted rate	6.5%	6.5%	2.2%	9.6%
Terminal rate	2/38 (5%)	3/42 (7%)	1/39 (3%)	1/14 (7%)
First incidence (days)	723	730 (T)	730 (T)	502
Poly-3 test	P=0.539	P=0.660	P=0.313N	P=0.475
All Organs: Benign Neoplasms				
Overall rate	34/50 (68%)	39/50 (78%)	45/50 (90%)	39/50 (78%)
Adjusted rate	70.2%	82.9%	91.8%	95.4%
Terminal rate	27/38 (71%)	35/42 (83%)	35/39 (90%)	14/14 (100%)
First incidence (days)	464	518	497	410
Poly-3 test	P<0.001	P=0.106	P=0.005	P<0.001
All Organs: Malignant Neoplasms				
Overall rate	29/50 (58%)	18/50 (36%)	30/50 (60%)	24/50 (48%)
Adjusted rate	58.5%	37.5%	62.0%	66.5%
Terminal rate	18/38 (47%)	12/42 (29%)	21/39 (54%)	6/14 (43%)
First incidence (days)	464	518	497	494
Poly-3 test	P=0.048	P=0.029N	P=0.441	P=0.299
All Organs: Benign or Malignant Neoplasms				
Overall rate	45/50 (90%)	43/50 (86%)	49/50 (98%)	41/50 (82%)
Adjusted rate	90.0%	89.6%	100.0%	98.3%
Terminal rate	33/38 (87%)	37/42 (88%)	39/39 (100%)	14/14 (100%)
First incidence (days)	464	518	497	410
Poly-3 test	P=0.014	P=0.604N	P=0.034	P=0.106

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

Table A4a
Historical Incidence of Forestomach Neoplasms in Control Male B6C3F$_1$ Mice

Study	Incidence in Controls		
	Squamous Cell Papilloma	Squamous Cell Carcinoma	Squamous Cell Papilloma or Carcinoma
Historical Incidence in Controls Given NTP-2000 Diet[a]			
Acrylonitrile (gavage)	3/50	0/50	3/50
Citral (feed)	0/100	0/100	0/100
p,p′-Dichlorodiphenyl sulfone (feed)	0/50	0/50	0/50
Indium phosphide (inhalation)	1/50	1/50	2/50
60-Hz Magnetic fields (whole body exposure)	0/100	0/100	0/100
Methacrylonitrile (gavage)	1/49	0/49	1/49
o-Nitrotoluene (feed)	0/60	0/60	0/60
p-Nitrotoluene (feed)	1/50	0/50	1/50
Riddelliine (gavage)	1/50	0/50	1/50
Sodium nitrite (drinking water)	1/50	0/50	1/50
Vanadium pentoxide (inhalation)	2/50	0/50	2/50
Overall Historical Incidence in Controls Given NTP-2000 Diet			
Total (%)	10/659 (1.5%)	1/659 (0.2%)	11/659 (1.7%)
Mean ± standard deviation	1.8% ± 1.9%	0.2% ± 0.6%	2.0% ± 2.0%
Range	0%-6%	0%-2%	0%-6%
Historical Incidence in Water Gavage Controls Given NIH-07 Diet at Battelle Columbus Laboratories[b]			
Scopolamine hydrobromide trihydrate	0/50	0/50	0/50

[a] Data as of 20 December 2000
[b] Data as of 23 December 1999

Table A4b
Historical Incidence of Harderian Gland Neoplasms in Control Male B6C3F$_1$ Mice

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence in Controls Given NTP-2000 Diet[a]			
Acrylonitrile (gavage)	5/50	1/50	6/50
Citral (feed)	4/100	0/100	4/100
p,p′-Dichlorodiphenyl sulfone (feed)	5/50	0/50	5/50
Indium phosphide (inhalation)	1/50	0/50	1/50
60-Hz Magnetic fields (whole body exposure)	12/100	0/100	12/100
Methacrylonitrile (gavage)	3/49	0/49	3/49
o-Nitrotoluene (feed)	4/60	1/60	5/60
p-Nitrotoluene (feed)	3/50	0/50	3/50
Riddelliine (gavage)	4/50	2/50	6/50
Sodium nitrite (drinking water)	4/50	0/50	4/50
Vanadium pentoxide (inhalation)	8/50	0/50	8/50
Overall Historical Incidence in Controls Given NTP-2000 Diet			
Total (%)	53/659 (8.0%)	4/659 (0.6%)	57/659 (8.7%)
Mean ± standard deviation	8.1% ± 3.9%	0.7% ± 1.3%	8.8% ± 4.1%
Range	2%-16%	0%-4%	2%-16%
Historical Incidence in Water Gavage Controls Given NIH-07 Diet at Battelle Columbus Laboratories[b]			
Scopolamine hydrobromide trihydrate	3/50 (6.0%)	0/50	3/50 (6.0%)

[a] Data as of 20 December 2000
[b] Data as of 23 December 1999

Table A4c
Historical Incidence of Hepatocellular Neoplasms in Control Male B6C3F$_1$ Mice

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence in Controls Given NTP-2000 Diet[a]			
Acrylonitrile (gavage)	23/50	14/50	32/50
Citral (feed)	20/100	13/100	28/100
p,p′-Dichlorodiphenyl sulfone (feed)	6/50	9/50	15/50
Indium phosphide (inhalation)	17/50	11/50	26/50
60-Hz Magnetic fields (whole body exposure)	30/100	19/100	46/100
Methacrylonitrile (gavage)	17/49	13/49	24/49
o-Nitrotoluene (feed)	18/60	12/60	27/60
p-Nitrotoluene (feed)	14/50	8/50	20/50
Riddelliine (gavage)	16/50	23/50	36/50
Sodium nitrite (drinking water)	19/50	9/50	24/50
Vanadium pentoxide (inhalation)	15/50	14/50	26/50
Overall Historical Incidence in Controls Given NTP-2000 Diet			
Total (%)	195/659 (29.6%)	145/659 (22.0%)	304/659 (46.1%)
Mean ± standard deviation	30.4% ± 8.9%	23.1% ± 9.0%	47.8% ± 12.9%
Range	12%-46%	13%-46%	28%-72%
Historical Incidence in Water Gavage Controls Given NIH-07 Diet at Battelle Columbus Laboratories[b]			
Scopolamine hydrobromide trihydrate	26/50 (52.0%)	6/50 (12.0%)	30/50 (60.0%)

[a] Data as of 20 December 2000
[b] Data as of 23 December 1999

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Acrylonitrile[a]

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths		2		
Moribund	1	3	3	4
Natural deaths	11	3	8	32
Survivors				
Terminal sacrifice	38	42	39	14
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation, suppurative		1 (2%)		
Perforation		2 (4%)		
Gallbladder	(50)	(49)	(50)	(48)
Cyst				1 (2%)
Intestine large, cecum	(50)	(50)	(50)	(50)
Inflammation, chronic active			1 (2%)	
Ulcer			1 (2%)	
Intestine small, duodenum	(50)	(50)	(50)	(50)
Ulcer		1 (2%)		
Intestine small, jejunum	(50)	(50)	(50)	(50)
Inflammation, chronic active			1 (2%)	
Peyer's patch, hyperplasia, lymphoid	2 (4%)	1 (2%)		
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	2 (4%)	5 (10%)	1 (2%)
Basophilic focus	1 (2%)	5 (10%)	5 (10%)	4 (8%)
Clear cell focus	19 (38%)	24 (48%)	16 (32%)	4 (8%)
Eosinophilic focus	5 (10%)	5 (10%)	10 (20%)	4 (8%)
Fibrosis			1 (2%)	1 (2%)
Hematopoietic cell proliferation	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Infarct	2 (4%)	1 (2%)	2 (4%)	
Inflammation, chronic active	25 (50%)	33 (66%)	33 (66%)	11 (22%)
Mineralization		1 (2%)	1 (2%)	
Mixed cell focus	9 (18%)	13 (26%)	13 (26%)	3 (6%)
Necrosis	4 (8%)	10 (20%)	5 (10%)	
Pigmentation		4 (8%)	3 (6%)	
Vacuolization cytoplasmic	18 (36%)	30 (60%)	23 (46%)	17 (34%)
Centrilobular, degeneration	1 (2%)			
Mesentery	(8)	(6)	(8)	(3)
Artery, inflammation, chronic active			1 (13%)	
Artery, necrosis, fibrinoid			1 (13%)	
Fat, inflammation, chronic active	1 (13%)			
Fat, mineralization			2 (25%)	
Fat, necrosis	2 (25%)	3 (50%)	4 (50%)	2 (67%)
Oral mucosa	(2)		(4)	(1)
Gingival, foreign body	2 (100%)		3 (75%)	
Gingival, inflammation, suppurative	2 (100%)		4 (100%)	1 (100%)
Pancreas	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell	1 (2%)	2 (4%)	1 (2%)	
Inflammation, chronic active	1 (2%)			
Acinus, atrophy		1 (2%)		1 (2%)

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

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Alimentary System (continued)				
Salivary glands	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell	35 (70%)	34 (68%)	36 (72%)	23 (46%)
Duct, cyst			1 (2%)	
Stomach, forestomach	(50)	(50)	(50)	(50)
Hyperkeratosis, diffuse			3 (6%)	6 (12%)
Hyperkeratosis, focal	2 (4%)	3 (6%)	4 (8%)	6 (12%)
Hyperplasia				1 (2%)
Inflammation, chronic active			1 (2%)	
Inflammation, chronic active, focal	1 (2%)	2 (4%)		2 (4%)
Inflammation, chronic active, multifocal		1 (2%)	1 (2%)	
Ulcer		1 (2%)	1 (2%)	1 (2%)
Ulcer, multifocal		2 (4%)	1 (2%)	
Epithelium, hyperplasia, focal	2 (4%)	4 (8%)	8 (16%)	9 (18%)
Stomach, glandular	(50)	(50)	(50)	(50)
Hyperplasia, focal	1 (2%)	1 (2%)		1 (2%)
Inflammation, chronic active		2 (4%)		
Mineralization			1 (2%)	
Ulcer	1 (2%)			
Glands, ectasia	2 (4%)	7 (14%)	11 (22%)	4 (8%)
Tooth	(9)	(5)	(4)	(3)
Inflammation, suppurative			1 (25%)	
Malformation	9 (100%)	5 (100%)	3 (75%)	2 (67%)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Aorta, mineralization			1 (2%)	
Heart	(50)	(50)	(50)	(50)
Inflammation, chronic active	1 (2%)	3 (6%)	2 (4%)	1 (2%)
Inflammation, suppurative			1 (2%)	
Mineralization	2 (4%)			1 (2%)
Valve, inflammation, chronic active			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Hypertrophy	19 (38%)	21 (42%)	19 (38%)	2 (4%)
Subcapsular, cyst multilocular	1 (2%)			
Subcapsular, hyperplasia	45 (90%)	43 (86%)	40 (80%)	36 (72%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)	1 (2%)	
Parathyroid gland	(42)	(44)	(34)	(41)
Cyst	2 (5%)			1 (2%)
Pituitary gland	(49)	(46)	(47)	(47)
Pars distalis, cyst	3 (6%)	2 (4%)	3 (6%)	2 (4%)
Thyroid gland	(50)	(50)	(50)	(50)
Follicle, cyst	3 (6%)	3 (6%)	5 (10%)	1 (2%)
General Body System				
None				

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			1 (2%)
Granuloma sperm	1 (2%)	1 (2%)		
Infiltration cellular, mononuclear cell	42 (84%)	31 (62%)	38 (76%)	16 (32%)
Inflammation, chronic active	2 (4%)		1 (2%)	
Inflammation, suppurative			1 (2%)	
Mineralization	1 (2%)			
Preputial gland	(49)	(50)	(50)	(50)
Hyperplasia, basal cell			1 (2%)	
Infiltration cellular, mononuclear cell	11 (22%)	13 (26%)	11 (22%)	13 (26%)
Inflammation, chronic active	8 (16%)	3 (6%)	8 (16%)	2 (4%)
Bilateral, inflammation, chronic active	1 (2%)			
Bilateral, duct, ectasia	4 (8%)	1 (2%)	2 (4%)	1 (2%)
Duct, ectasia	13 (27%)	10 (20%)	20 (40%)	15 (30%)
Prostate	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell	36 (72%)	31 (62%)	29 (58%)	22 (44%)
Inflammation, chronic active	2 (4%)	2 (4%)		
Metaplasia, mucous				1 (2%)
Mineralization			1 (2%)	
Seminal vesicle	(50)	(50)	(50)	(50)
Atrophy				1 (2%)
Inflammation, chronic active	1 (2%)			
Testes	(50)	(50)	(50)	(50)
Inflammation, suppurative			1 (2%)	
Mineralization	2 (4%)	1 (2%)	2 (4%)	
Necrosis	1 (2%)			
Germinal epithelium, degeneration	2 (4%)	1 (2%)	3 (6%)	1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Angiectasis			2 (4%)	
Atrophy			1 (2%)	
Inflammation, suppurative			1 (2%)	
Myeloid cell, hyperplasia		1 (2%)	4 (8%)	2 (4%)
Lymph node	(6)	(4)	(6)	(5)
Mediastinal, hyperplasia, lymphoid		1 (25%)	1 (17%)	
Pancreatic, hyperplasia, lymphoid				1 (20%)
Lymph node, mandibular	(49)	(47)	(47)	(47)
Hematopoietic cell proliferation		1 (2%)		
Hyperplasia, lymphoid	1 (2%)	1 (2%)	5 (11%)	4 (9%)
Lymph node, mesenteric	(48)	(48)	(49)	(48)
Angiectasis				1 (2%)
Atrophy				1 (2%)
Ectasia				2 (4%)
Hematopoietic cell proliferation	1 (2%)			2 (4%)
Hyperplasia, lymphoid	4 (8%)	1 (2%)		
Hyperplasia, plasma cell		1 (2%)		
Inflammation, chronic active	1 (2%)			1 (2%)

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Hematopoietic System (continued)				
Spleen	(50)	(50)	(50)	(50)
Depletion cellular	1 (2%)			
Fibrosis				1 (2%)
Hematopoietic cell proliferation	17 (34%)	7 (14%)	16 (32%)	12 (24%)
Hyperplasia, lymphoid	2 (4%)			
Lymphoid follicle, depletion cellular				1 (2%)
Red pulp, depletion cellular		1 (2%)		
Thymus	(47)	(47)	(44)	(46)
Atrophy	7 (15%)	4 (9%)	7 (16%)	4 (9%)
Inflammation, chronic active		1 (2%)		
Thymocyte, necrosis		1 (2%)		3 (7%)
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell	1 (2%)		1 (2%)	
Inflammation, chronic active			1 (2%)	
Ulcer			1 (2%)	
Subcutaneous tissue, infiltration cellular, mast cell		1 (2%)		
Subcutaneous tissue, inflammation, suppurative			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis		1 (2%)		1 (2%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Cyst epithelial inclusion			1 (2%)	
Hemorrhage		2 (4%)		
Inflammation, chronic active		1 (2%)		
Thrombosis		1 (2%)		
Vacuolization cytoplasmic		1 (2%)		
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Erythrophagocytosis	1 (2%)		2 (4%)	
Inflammation, chronic active	4 (8%)	1 (2%)	3 (6%)	
Inflammation, granulomatous				1 (2%)
Inflammation, suppurative		2 (4%)		1 (2%)
Metaplasia, osseous	1 (2%)			
Mineralization			1 (2%)	
Necrosis	1 (2%)			
Pigmentation	1 (2%)		2 (4%)	
Alveolar epithelium, hyperplasia	4 (8%)	3 (6%)	5 (10%)	6 (12%)
Alveolus, infiltration cellular, histiocyte	1 (2%)		4 (8%)	
Artery, mediastinum, mineralization			1 (2%)	
Mediastinum, hemorrhage		1 (2%)		
Mediastinum, inflammation, chronic active	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Foreign body			1 (2%)	
Inflammation, suppurative	1 (2%)		1 (2%)	
Nasolacrimal duct, inflammation, suppurative	4 (8%)	1 (2%)	4 (8%)	7 (14%)

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Special Senses System				
Eye		(1)	(6)	(3)
Atrophy		1 (100%)	4 (67%)	2 (67%)
Synechia			2 (33%)	2 (67%)
Cornea, inflammation, chronic active			3 (50%)	3 (100%)
Cornea, sclera, inflammation, chronic active			1 (17%)	
Lens, cataract		1 (100%)	3 (50%)	2 (67%)
Retina, atrophy			1 (17%)	
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	4 (8%)	7 (14%)	4 (8%)
Infiltration cellular, mononuclear cell	33 (66%)	25 (50%)	26 (52%)	14 (28%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet			1 (2%)	
Infarct	5 (10%)	3 (6%)	3 (6%)	5 (10%)
Inflammation, chronic active	2 (4%)	3 (6%)	3 (6%)	2 (4%)
Inflammation, suppurative		1 (2%)		
Metaplasia, osseous	5 (10%)	2 (4%)	5 (10%)	3 (6%)
Mineralization	23 (46%)	28 (56%)	32 (64%)	18 (36%)
Necrosis		1 (2%)		
Nephropathy	41 (82%)	39 (78%)	42 (84%)	19 (38%)
Artery, inflammation, chronic active		1 (2%)		
Cortex, cyst	10 (20%)	13 (26%)	13 (26%)	4 (8%)
Glomerulus, thrombosis			1 (2%)	
Pelvis, inflammation, suppurative		1 (2%)		
Renal tubule, casts protein			1 (2%)	
Renal tubule, hyperplasia	5 (10%)	5 (10%)	4 (8%)	

APPENDIX B
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR GAVAGE STUDY OF ACRYLONITRILE

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Table B1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Acrylonitrile[a]

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death				1
Moribund	5	3	1	1
Natural deaths	6	15	10	25
Survivors				
Died last week of study				1
Terminal sacrifice	39	32	39	22
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, colon	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	
Leiomyosarcoma			1 (2%)	
Intestine large, rectum	(50)	(50)	(49)	(50)
Intestine large, cecum	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	
Intestine small, duodenum	(50)	(50)	(49)	(50)
Histiocytic sarcoma			1 (2%)	
Intestine small, jejunum	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	
Intestine small, ileum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)	
Hemangiosarcoma	2 (4%)	1 (2%)	1 (2%)	
Hepatocellular carcinoma	7 (14%)	3 (6%)	6 (12%)	2 (4%)
Hepatocellular adenoma	8 (16%)	10 (20%)	10 (20%)	9 (18%)
Hepatocellular adenoma, multiple	6 (12%)	4 (8%)	5 (10%)	1 (2%)
Hepatocholangiocarcinoma				1 (2%)
Histiocytic sarcoma	1 (2%)		1 (2%)	
Lipoma			1 (2%)	
Sarcoma, metastatic, mesentery			1 (2%)	
Squamous cell carcinoma, metastatic, stomach, forestomach		1 (2%)	1 (2%)	7 (14%)
Mesentery	(9)	(10)	(10)	(10)
Fibrosarcoma, metastatic, skin		1 (10%)		
Hemangiosarcoma	1 (11%)		1 (10%)	
Sarcoma			1 (10%)	
Sarcoma, metastatic, skin	1 (11%)			1 (10%)
Fat, squamous cell carcinoma, metastatic, stomach, forestomach				2 (20%)
Pancreas	(50)	(50)	(50)	(50)
Sarcoma, metastatic, skin				1 (2%)
Salivary glands	(50)	(50)	(49)	(50)
Sarcoma, metastatic, skin	1 (2%)			
Stomach, forestomach	(50)	(50)	(50)	(50)
Fibrosarcoma, metastatic, skin		1 (2%)		
Squamous cell carcinoma		1 (2%)	1 (2%)	11 (22%)
Squamous cell papilloma	2 (4%)	6 (12%)	20 (40%)	10 (20%)
Squamous cell papilloma, multiple	1 (2%)		4 (8%)	9 (18%)

Table B1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Alimentary System (continued)				
Stomach, glandular	(50)	(50)	(50)	(50)
Fibrosarcoma, metastatic, skin		1 (2%)		
Histiocytic sarcoma	1 (2%)		1 (2%)	
Squamous cell carcinoma, metastatic, stomach, forestomach				3 (6%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)	
Fibrosarcoma, metastatic, skin				1 (2%)
Sarcoma, metastatic, skin	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)			
Bilateral, histiocytic sarcoma			1 (2%)	
Subcapsular, adenoma, multiple				1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign		1 (2%)		
Bilateral, pheochromocytoma benign			1 (2%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma		1 (2%)	1 (2%)	
Pituitary gland	(48)	(49)	(48)	(49)
Meningioma malignant, metastatic, brain	1 (2%)			
Pars distalis, adenoma	2 (4%)	4 (8%)	4 (8%)	2 (4%)
Thyroid gland	(50)	(50)	(49)	(50)
Follicular cell, adenoma	2 (4%)	1 (2%)		
General Body System				
None				
Genital System				
Clitoral gland	(50)	(48)	(49)	(48)
Ovary	(50)	(50)	(50)	(50)
Cystadenoma	8 (16%)	4 (8%)	2 (4%)	3 (6%)
Granulosa cell tumor malignant			1 (2%)	
Granulosa cell tumor benign			3 (6%)	1 (2%)
Hemangioma			1 (2%)	2 (4%)
Histiocytic sarcoma	1 (2%)			
Luteoma		1 (2%)		
Oviduct		(1)		(1)
Uterus	(50)	(50)	(50)	(50)
Hemangioma		1 (2%)		2 (4%)
Histiocytic sarcoma	1 (2%)			
Leiomyoma		1 (2%)		
Polyp stromal		1 (2%)	3 (6%)	

Table B1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	
Lymph node	(37)	(32)	(30)	(25)
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung			1 (3%)	
Mediastinal, histiocytic sarcoma	1 (3%)			
Mediastinal, liposarcoma, metastatic, skin			1 (3%)	
Mediastinal, sarcoma, metastatic, mesentery			1 (3%)	
Pancreatic, histiocytic sarcoma	1 (3%)			
Lymph node, mandibular	(49)	(49)	(47)	(50)
Carcinoma, metastatic, harderian gland			1 (2%)	1 (2%)
Histiocytic sarcoma	1 (2%)			
Lymph node, mesenteric	(50)	(49)	(50)	(47)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)	
Fibrosarcoma, metastatic, skin				1 (2%)
Hemangiosarcoma		1 (2%)		
Histiocytic sarcoma	1 (2%)		1 (2%)	
Sarcoma, metastatic, skin				1 (2%)
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)		
Histiocytic sarcoma	1 (2%)		1 (2%)	
Squamous cell carcinoma, metastatic, stomach, forestomach				1 (2%)
Thymus	(50)	(50)	(47)	(50)
Fibrosarcoma, metastatic, skin				1 (2%)
Histiocytic sarcoma			1 (2%)	
Thymoma benign		1 (2%)		
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Carcinoma			1 (2%)	
Fibroadenoma				1 (2%)
Skin	(50)	(50)	(50)	(50)
Basal cell carcinoma		1 (2%)		
Subcutaneous tissue, alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Subcutaneous tissue, fibrosarcoma	2 (4%)	2 (4%)	3 (6%)	2 (4%)
Subcutaneous tissue, fibrosarcoma, multiple		1 (2%)		
Subcutaneous tissue, fibrous histiocytoma		1 (2%)		
Subcutaneous tissue, hemangiosarcoma	1 (2%)			
Subcutaneous tissue, histiocytic sarcoma	1 (2%)			
Subcutaneous tissue, sarcoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Musculoskeletal System				
Skeletal muscle	(3)	(3)	(2)	(1)
Histiocytic sarcoma	1 (33%)			
Sarcoma, metastatic, mesentery			1 (50%)	
Sarcoma, metastatic, skin	1 (33%)			

Table B1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Nervous System				
Brain	(50)	(50)	(50)	(50)
Meningioma malignant	1 (2%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	4 (8%)	1 (2%)	8 (16%)	5 (10%)
Alveolar/bronchiolar carcinoma	2 (4%)	5 (10%)	6 (12%)	4 (8%)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Basal cell carcinoma, metastatic, skin		1 (2%)		
Carcinoma, metastatic, harderian gland	1 (2%)		3 (6%)	1 (2%)
Fibrosarcoma, metastatic, skin			1 (2%)	1 (2%)
Hepatocellular carcinoma, metastatic, liver		1 (2%)	2 (4%)	
Hepatocholangiocarcinoma, metastatic, liver				1 (2%)
Histiocytic sarcoma	1 (2%)		1 (2%)	
Sarcoma, metastatic, skin	1 (2%)		1 (2%)	
Squamous cell carcinoma, metastatic, stomach, forestomach		1 (2%)		1 (2%)
Bronchus, adenoma	1 (2%)			
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	1 (2%)	
Mediastinum, fibrosarcoma, metastatic, skin		1 (2%)		
Mediastinum, sarcoma, metastatic, skin	1 (2%)		1 (2%)	
Nose	(50)	(50)	(50)	(50)
Special Senses System				
Harderian gland	(49)	(50)	(49)	(49)
Adenoma	8 (16%)	10 (20%)	21 (43%)	19 (39%)
Carcinoma	1 (2%)		3 (6%)	2 (4%)
Bilateral, adenoma	2 (4%)		4 (8%)	4 (8%)
Zymbal's gland	(1)			
Carcinoma	1 (100%)			
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)		1 (2%)	
Histiocytic sarcoma	1 (2%)		1 (2%)	
Capsule, squamous cell carcinoma, metastatic, stomach, forestomach				1 (2%)
Renal tubule, adenoma		1 (2%)	1 (2%)	1 (2%)
Urinary bladder	(50)	(49)	(50)	(50)
Histiocytic sarcoma	1 (2%)			

Table B1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)		1 (2%)	
Lymphoma malignant	4 (8%)	5 (10%)	7 (14%)	6 (12%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	39	41	50	40
Total primary neoplasms	68	71	123	99
Total animals with benign neoplasms	29	33	45	33
Total benign neoplasms	44	48	89	70
Total animals with malignant neoplasms	24	22	30	24
Total malignant neoplasms	24	23	34	29
Total animals with metastatic neoplasms	4	5	10	12
Total metastatic neoplasms	11	10	21	26

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

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Acrylonitrile: Vehicle Control

Number of Days on Study	338	562	638	653	659	673	677	701	704	708	708	729	729	729	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	208	203	211	222	214	249	250	246	240	202	225	201	205	206	207	210	216	218	221	228	233	234	235	241	243
Alimentary System																									
Esophagus																									
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																X									
Hepatocellular carcinoma						X							X									X			
Hepatocellular adenoma												X			X	X	X		X				X		
Hepatocellular adenoma, multiple														X							X				
Histiocytic sarcoma			X																						
Mesentery				+	+											+						+			
Hemangiosarcoma																									
Sarcoma, metastatic, skin					X																				
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skin					X																				
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Squamous cell papilloma, multiple																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skin					X																				
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	M	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Meningioma malignant, metastatic, brain								X																	
Pars distalis, adenoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma														X								X			
General Body System																									
None																									

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Acrylonitrile: Vehicle Control

Number of Days on Study	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	244	204	212	213	215	217	219	224	227	229	231	236	238	242	245	247	209	220	223	226	230	232	237	239	248		Total Tissues/ Tumors
Alimentary System																											
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Hemangiosarcoma													X														2
Hepatocellular carcinoma					X							X								X				X			7
Hepatocellular adenoma			X								X																8
Hepatocellular adenoma, multiple														X				X					X	X			6
Histiocytic sarcoma																											1
Mesentery			+						+	+						+	+										9
Hemangiosarcoma			X																								1
Sarcoma, metastatic, skin																											1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Sarcoma, metastatic, skin																											1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Squamous cell papilloma																							X	X			2
Squamous cell papilloma, multiple												X															1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Histiocytic sarcoma																											1
Cardiovascular System																											
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Sarcoma, metastatic, skin																											1
Endocrine System																											
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Histiocytic sarcoma																											1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	M	+		43
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+		48
Meningioma malignant, metastatic, brain																											1
Pars distalis, adenoma	X															X											2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Follicular cell, adenoma																											2
General Body System																											
None																											

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Acrylonitrile: Vehicle Control

Number of Days on Study	338	562	638	653	659	673	677	701	704	708	708	729	729	729	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	208	203	211	222	214	249	250	246	240	202	225	201	205	206	207	210	216	218	221	228	233	234	235	241	243
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma					X											X					X				
Histiocytic sarcoma			X																						
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+				+		+	+			+	+	+		+		+	+	+	+	+	+
Mediastinal, histiocytic sarcoma			X																						
Pancreatic, histiocytic sarcoma			X																						
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, alveolar/bronchiolar carcinoma, metastatic, lung										X															
Subcutaneous tissue, fibrosarcoma				X																					
Subcutaneous tissue, hemangiosarcoma																									X
Subcutaneous tissue, histiocytic sarcoma			X																						
Subcutaneous tissue, sarcoma					X																				
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle			+		+											+									
Histiocytic sarcoma			X																						
Sarcoma, metastatic, skin					X																				
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Meningioma malignant								X																	

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Acrylonitrile: Vehicle Control

Number of Days on Study	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	244	204	212	213	215	217	219	224	227	229	231	236	238	242	245	247	209	220	223	226	230	232	237	239	248	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cystadenoma			X		X		X									X	X									8
Histiocytic sarcoma																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+		+	+	+	+	+		+	+		+	+	+	+	+	+	+	+	+	+	+		+		37
Mediastinal, histiocytic sarcoma																										1
Pancreatic, histiocytic sarcoma																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	49
Histiocytic sarcoma																										1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Subcutaneous tissue, fibrosarcoma								X																		2
Subcutaneous tissue, hemangiosarcoma																										1
Subcutaneous tissue, histiocytic sarcoma																										1
Subcutaneous tissue, sarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										3
Histiocytic sarcoma																										1
Sarcoma, metastatic, skin																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Meningioma malignant																										1

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Acrylonitrile: Vehicle Control

Number of Days on Study	338	562	638	653	659	673	677	701	704	708	708	729	729	729	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	208	203	211	222	214	249	250	246	240	202	225	201	205	206	207	210	216	218	221	228	233	234	235	241	243
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma								X									X								
Alveolar/bronchiolar carcinoma							X			X															
Alveolar/bronchiolar carcinoma, metastatic, lung										X															
Carcinoma, metastatic, harderian gland																				X					
Histiocytic sarcoma			X																						
Sarcoma, metastatic, skin					X																				
Bronchus, adenoma																									
Mediastinum, sarcoma, metastatic, skin					X																				
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Harderian gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma	X												X								X		X		
Carcinoma																				X					
Bilateral, adenoma																X									
Zymbal's gland		+																							
Carcinoma		X																							
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung										X															
Histiocytic sarcoma			X																						
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Lymphoma malignant																							X		

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Acrylonitrile: Vehicle Control

Number of Days on Study	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	244	204	212	213	215	217	219	224	227	229	231	236	238	242	245	247	209	220	223	226	230	232	237	239	248	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma	X						X																			4
Alveolar/bronchiolar carcinoma																										2
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Carcinoma, metastatic, harderian gland																										1
Histiocytic sarcoma																										1
Sarcoma, metastatic, skin																										1
Bronchus, adenoma																			X							1
Mediastinum, sarcoma, metastatic, skin																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma				X						X						X				X						8
Carcinoma																										1
Bilateral, adenoma											X															2
Zymbal's gland																										1
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Histiocytic sarcoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymphoma malignant																X	X	X								4

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Acrylonitrile: 2.5 mg/kg

Number of Days on Study	354	424	468	563	633	640	643	647	648	653	665	673	683	688	694	696	721	724	729	729	729	729	729	729	729
Carcass ID Number	258	251	267	300	283	269	297	289	277	252	291	284	293	287	279	278	260	275	254	256	257	259	261	262	272
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Hepatocellular carcinoma																							X		
Hepatocellular adenoma						X								X				X					X		
Hepatocellular adenoma, multiple													X							X		X			
Squamous cell carcinoma, metastatic, stomach, forestomach											X														
Mesentery			+		+										+					+			+		
Fibrosarcoma, metastatic, skin															X										
Oral mucosa																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin															X										
Squamous cell carcinoma											X														
Squamous cell papilloma														X						X					
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin															X										
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma				X																					
Parathyroid gland	+	+	+	M	M	M	+	+	+	+	M	M	+	+	M	M	+	M	+	+	+	+	M	M	+
Pituitary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma										X							X					X			
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									
General Body System																									
None																									

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Acrylonitrile: 2.5 mg/kg

Number of Days on Study	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	
Carcass ID Number	276	286	288	290	292	294	295	298	263	264	265	266	268	271	273	280	281	282	285	296	299	253	255	270	274	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																X										1
Hepatocellular carcinoma													X				X									3
Hepatocellular adenoma	X			X				X									X	X						X		10
Hepatocellular adenoma, multiple															X											4
Squamous cell carcinoma, metastatic, stomach, forestomach																										1
Mesentery					+	+								+								+			+	10
Fibrosarcoma, metastatic, skin																										1
Oral mucosa						+																				1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, skin																										1
Squamous cell carcinoma																										1
Squamous cell papilloma					X		X														X		X			6
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, skin																										1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign																			X							1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	39
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma	X																									4
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																								X		1
General Body System																										
None																										

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Acrylonitrile: 2.5 mg/kg

Number of Days on Study	354	424	468	563	633	640	643	647	648	653	665	673	683	688	694	696	721	724	729	729	729	729	729	729	729	729
Carcass ID Number	258	251	267	300	283	269	297	289	277	252	291	284	293	287	279	278	260	275	254	256	257	259	261	262	272
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma																					X				
Luteoma																									
Oviduct				+																					
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma				X																					
Leiomyoma																									
Polyp stromal																		X							
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node				+	+	+	+	+	+	+		+		+		+			+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymoma benign																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma																		X							
Subcutaneous tissue, fibrosarcoma																X									
Subcutaneous tissue, fibrosarcoma, multiple															X										
Subcutaneous tissue, fibrous histiocytoma						X																			
Subcutaneous tissue, sarcoma																					X				
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle												+			+										
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Acrylonitrile: 2.5 mg/kg

Number of Days on Study	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	
Carcass ID Number	276	286	288	290	292	294	295	298	263	264	265	266	268	271	273	280	281	282	285	296	299	253	255	270	274	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cystadenoma			X			X			X																	4
Luteoma																X										1
Oviduct																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma																										1
Leiomyoma										X																1
Polyp stromal																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node			+	+	+	+	+	+			+	+		+	+		+			+			+	+	+	32
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma								X																		1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																				X						1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymoma benign												X														1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell carcinoma																										1
Subcutaneous tissue, fibrosarcoma											X															2
Subcutaneous tissue, fibrosarcoma, multiple																										1
Subcutaneous tissue, fibrous histiocytoma																										1
Subcutaneous tissue, sarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle														+												3
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Acrylonitrile: 2.5 mg/kg

Number of Days on Study	354	424	468	563	633	640	643	644	648	653	665	673	683	688	694	696	721	724	729	729	729	729	729	729	729	729
Carcass ID Number	258	251	267	300	283	269	297	289	277	252	291	284	293	287	279	278	260	275	254	256	257	259	261	262	272
Respiratory System																										
Larynx														+												
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma					X																					
Alveolar/bronchiolar carcinoma								X					X	X		X										
Alveolar/bronchiolar carcinoma, metastatic, lung								X																		
Basal cell carcinoma, metastatic, skin																		X								
Hepatocellular carcinoma, metastatic, liver																							X			
Squamous cell carcinoma, metastatic, stomach, forestomach											X															
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung								X																		
Mediastinum, fibrosarcoma, metastatic, skin															X											
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																										
Eye																										
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma				X										X										X		
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																										
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant	X			X																				X		

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Acrylonitrile: 2.5 mg/kg

Number of Days on Study	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	
Carcass ID Number	276	286	288	290	292	294	295	298	263	264	265	266	268	271	273	280	281	282	285	296	299	253	255	270	274		Total Tissues/ Tumors
Respiratory System																											
Larynx																											1
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Alveolar/bronchiolar adenoma																											1
Alveolar/bronchiolar carcinoma			X																								5
Alveolar/bronchiolar carcinoma, metastatic, lung																											1
Basal cell carcinoma, metastatic, skin																											1
Hepatocellular carcinoma, metastatic, liver																											1
Squamous cell carcinoma, metastatic, stomach, forestomach																											1
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																											1
Mediastinum, fibrosarcoma, metastatic, skin																											1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Special Senses System																											
Eye																	+										1
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Adenoma					X					X	X	X				X	X			X							10
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Renal tubule, adenoma			X																								1
Urinary bladder	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Lymphoma malignant														X											X		5

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Acrylonitrile: 10 mg/kg

Number of Days on Study	190	516	639	645	646	695	708	711	715	716	721	729	729	729	729	729	729	729	729	729	729	729	729	729	730
Carcass ID Number	316	317	318	337	302	345	341	340	314	329	309	306	308	320	325	327	330	332	334	335	338	339	344	348	301
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma		X																							
Leiomyosarcoma												X													
Intestine large, rectum	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma		X																							
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma		X																							
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma		X																							
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung				X																					
Hemangiosarcoma																									
Hepatocellular carcinoma									X																X
Hepatocellular adenoma										X			X	X								X			
Hepatocellular adenoma, multiple															X				X	X					
Histiocytic sarcoma		X																							
Lipoma																									
Sarcoma, metastatic, mesentery						X																			
Squamous cell carcinoma, metastatic, stomach, forestomach			X																						
Mesentery					+	+																			
Hemangiosarcoma																									
Sarcoma						X																			
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma			X																						
Squamous cell papilloma										X	X	X						X			X		X	X	
Squamous cell papilloma, multiple														X											
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma		X																							
Tooth																								+	
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung				X																					

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Acrylonitrile: 10 mg/kg

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	
Carcass ID Number	303	307	310	311	312	313	315	321	323	328	331	333	342	443	446	449	500	404	305	319	322	324	326	336	347	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Leiomyosarcoma																										1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Intestine small, duodenum	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Hemangiosarcoma																				X						1
Hepatocellular carcinoma		X											X								X		X			6
Hepatocellular adenoma				X		X				X		X							X				X			10
Hepatocellular adenoma, multiple													X			X										5
Histiocytic sarcoma																										1
Lipoma			X																							1
Sarcoma, metastatic, mesentery																										1
Squamous cell carcinoma, metastatic, stomach, forestomach																										1
Mesentery		+		+	+	+		+			+		+							+						10
Hemangiosarcoma													X													1
Sarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma																										1
Squamous cell papilloma	X		X		X	X				X	X								X	X	X	X	X	X	X	20
Squamous cell papilloma, multiple							X									X		X								4
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Tooth																										1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Acrylonitrile: 10 mg/kg

Number of Days on Study	190	516	639	645	646	695	708	711	715	716	721	729	729	729	729	729	729	729	729	729	729	729	729	729	730
Carcass ID Number	316	317	318	337	302	345	341	340	314	329	309	306	308	320	325	327	330	332	334	335	338	339	344	348	301
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, histiocytic sarcoma		X																							
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, pheochromocytoma benign									X																
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																		X							
Parathyroid gland	M	+	+	+	+	+	+	+	M	+	M	M	M	M	M	+	M	+	M	+	+	M	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma					X												X								
Thyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma																					X				
Granulosa cell tumor malignant												X													
Granulosa cell tumor benign																	X								
Hemangioma																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal																	X				X				
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma		X																							
Lymph node				+	+	+	+	+			+	+	+		+	+	+	+	+		+	+	+		+
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung				X																					
Mediastinal, liposarcoma, metastatic, skin																X									
Mediastinal, sarcoma, metastatic, mesentery						X																			
Lymph node, mandibular	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, harderian gland																									
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung				X																					
Histiocytic sarcoma		X																							
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma		X																							
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Histiocytic sarcoma		X																							
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma														X											
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma					X		X		X																
Subcutaneous tissue, sarcoma																X									

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Acrylonitrile: 10 mg/kg

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	
Carcass ID Number	303	307	310	311	312	313	315	321	323	328	331	333	342	443	446	449	450	304	305	319	322	324	326	336	347	Total Tissues/ Tumors
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, histiocytic sarcoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Parathyroid gland	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	37
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma								X	X																	4
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cystadenoma				X																						2
Granulosa cell tumor malignant																										1
Granulosa cell tumor benign						X							X													3
Hemangioma											X															1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal																							X			3
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymph node			+	+		+	+		+	+			+	+	+	+					+		+	+		30
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Mediastinal, liposarcoma, metastatic, skin																										1
Mediastinal, sarcoma, metastatic, mesentery																										1
Lymph node, mandibular	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Carcinoma, metastatic, harderian gland							X																			1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Histiocytic sarcoma																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	47
Histiocytic sarcoma																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma																										3
Subcutaneous tissue, sarcoma																										1

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Acrylonitrile: 10 mg/kg

Number of Days on Study	190	516	639	645	646	695	708	711	715	716	721	729	729	729	729	729	729	729	729	729	729	729	729	729	730
Carcass ID Number	316	317	318	337	302	345	341	340	314	329	309	306	308	320	325	327	330	332	334	335	338	339	344	348	301
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle					+	+																			
Sarcoma, metastatic, mesentery						X																			
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma			X				X			X							X				X				
Alveolar/bronchiolar carcinoma				X															X						
Alveolar/bronchiolar carcinoma, metastatic, lung				X																					
Carcinoma, metastatic, harderian gland								X																	
Fibrosarcoma, metastatic, skin					X																				
Hepatocellular carcinoma, metastatic, liver																									
Histiocytic sarcoma		X																							
Sarcoma, metastatic, skin																X									
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung				X																					
Mediastinum, sarcoma, metastatic, skin																X									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye			+																						
Harderian gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma					X			X				X	X					X			X	X	X		
Carcinoma								X																	
Bilateral, adenoma			X													X				X					
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung				X																					
Histiocytic sarcoma		X																							
Renal tubule, adenoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma		X																							
Lymphoma malignant	X														X		X								

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Acrylonitrile: 10 mg/kg

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	
Carcass ID Number	303	307	310	311	312	313	315	321	323	328	331	333	342	343	346	349	350	304	305	319	322	324	326	336	347	Total Tissues/ Tumors
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										2
Sarcoma, metastatic, mesentery																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma									X								X								X	8
Alveolar/bronchiolar carcinoma			X		X	X																		X		6
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Carcinoma, metastatic, harderian gland							X												X							3
Fibrosarcoma, metastatic, skin																										1
Hepatocellular carcinoma, metastatic, liver													X								X					2
Histiocytic sarcoma																										1
Sarcoma, metastatic, skin																										1
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Mediastinum, sarcoma, metastatic, skin																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																										
Eye									+																	2
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma		X		X				X	X	X	X	X		X			X		X	X			X		X	21
Carcinoma							X												X							3
Bilateral, adenoma			X																							4
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Histiocytic sarcoma																										1
Renal tubule, adenoma															X											1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymphoma malignant			X						X					X		X										7

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Acrylonitrile: 20 mg/kg

Number of Days on Study	113	135	135	185	352	352	352	382	386	429	494	513	547	555	580	582	640	642	649	649	660	667	680	704	704
Carcass ID Number	376	382	385	373	368	369	370	351	357	362	391	396	366	400	361	378	352	359	363	392	355	372	374	386	398
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																							X		
Hepatocellular adenoma										X											X		X		
Hepatocellular adenoma, multiple																									
Hepatocholangiocarcinoma																									
Squamous cell carcinoma, metastatic, stomach, forestomach												X			X									X	X
Mesentery												+									+	+	+	+	
Sarcoma, metastatic, skin																							X		
Fat, squamous cell carcinoma, metastatic, stomach, forestomach												X										X			
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skin																							X		
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma												X			X				X		X			X	X
Squamous cell papilloma																				X	X	X			
Squamous cell papilloma, multiple														X									X		
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, stomach, forestomach															X										X
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin																				X					
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcapsular, adenoma, multiple																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	+	+	+	+	+	+	M	+	+	M	+	+	+	+	M	M	+	M	M	+	+	M	+	M
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																	X								
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Acrylonitrile: 20 mg/kg

Number of Days on Study	711	722	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	731	731	731	731	
Carcass ID Number	356	399	354	365	367	377	381	387	388	389	390	394	395	397	358	364	371	379	380	384	393	353	360	375	383	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma						X																				2
Hepatocellular adenoma						X								X		X			X		X		X			9
Hepatocellular adenoma, multiple																				X						1
Hepatocholangiocarcinoma							X																			1
Squamous cell carcinoma, metastatic, stomach, forestomach	X	X									X															7
Mesentery						+		+								+						+	+			10
Sarcoma, metastatic, skin																										1
Fat, squamous cell carcinoma, metastatic, stomach, forestomach																										2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, metastatic, skin																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma	X	X									X	X					X									11
Squamous cell papilloma					X	X		X	X					X				X			X					10
Squamous cell papilloma, multiple			X							X			X		X				X			X	X			9
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma, metastatic, stomach, forestomach		X																								3
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, skin																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcapsular, adenoma, multiple																			X							1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	M	+	+	+	+	+	M	+	M	M	M	M	+	+	M	+	+	+	+	+	+	+	+	+	34
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma							X																			2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
General Body System																										
None																										

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Acrylonitrile: 20 mg/kg

Number of Days on Study	113	135	135	185	352	352	352	382	386	429	494	513	547	555	580	582	640	642	649	649	660	667	680	704	704
Carcass ID Number	376	382	385	373	368	369	370	351	357	362	391	396	366	400	361	378	352	359	363	392	355	372	374	386	398
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma																									X
Granulosa cell tumor benign																									
Hemangioma																					X			X	
Oviduct																						+			
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																			X						
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node						+	+		+	+		+				+	+	+			+	+		+	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, harderian gland																									
Lymph node, mesenteric	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin																				X					
Sarcoma, metastatic, skin																							X		
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, stomach, forestomach												X													
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin																				X					
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																	X								
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma														X						X					
Subcutaneous tissue, sarcoma																							X		
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																						+			
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																						X			
Alveolar/bronchiolar carcinoma																							X		
Alveolar/bronchiolar carcinoma, metastatic, lung																									
Carcinoma, metastatic, harderian gland																									
Fibrosarcoma, metastatic, skin																				X					
Hepatocholangiocarcinoma, metastatic, liver																									
Squamous cell carcinoma, metastatic, stomach, forestomach																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Acrylonitrile: 20 mg/kg

Number of Days on Study	711	722	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	731	731	731	731	
Carcass ID Number	356	399	354	365	367	377	381	387	388	389	390	394	395	397	358	364	371	379	380	384	393	353	360	375	383	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cystadenoma								X													X					3
Granulosa cell tumor benign					X																					1
Hemangioma																										2
Oviduct																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma					X																					2
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node					+		+				+	+		+	+	+	+	+		+	+	+		+	+	25
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, harderian gland	X																									1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Fibrosarcoma, metastatic, skin																										1
Sarcoma, metastatic, skin																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma, metastatic, stomach, forestomach																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, skin																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibroadenoma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma																										2
Subcutaneous tissue, sarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma			X						X									X	X							5
Alveolar/bronchiolar carcinoma													X	X											X	4
Alveolar/bronchiolar carcinoma, metastatic, lung														X												1
Carcinoma, metastatic, harderian gland																	X									1
Fibrosarcoma, metastatic, skin																										1
Hepatocholangiocarcinoma, metastatic, liver							X																			1
Squamous cell carcinoma, metastatic, stomach, forestomach											X															1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Acrylonitrile: 20 mg/kg

Number of Days on Study	113	135	135	185	352	352	352	382	386	429	494	513	547	555	580	582	640	642	649	649	660	667	680	704	704
Carcass ID Number	376	382	385	373	368	369	370	351	357	362	391	396	366	400	361	378	352	359	363	392	355	372	374	386	398
Special Senses System																									
Eye					+																				
Harderian gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma													X			X				X	X			X	X
Carcinoma																									
Bilateral, adenoma																							X		
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Capsule, squamous cell carcinoma, metastatic, stomach, forestomach																								X	
Renal tubule, adenoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant										X								X				X			

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Acrylonitrile: 20 mg/kg

Number of Days on Study	711	722	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	731	731	731	731	
Carcass ID Number	356	399	354	365	367	377	381	387	388	389	390	394	395	397	358	364	371	379	380	384	393	353	360	375	383	Total Tissues/ Tumors
Special Senses System																										
Eye				+			+	+	+								+	+	+						+	9
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma		X	X	X		X	X		X					X	X				X		X	X		X	X	19
Carcinoma	X																X									2
Bilateral, adenoma								X										X					X			4
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Capsule, squamous cell carcinoma, metastatic, stomach, forestomach																										1
Renal tubule, adenoma			X																							1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant																X				X					X	6

Table B3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	10/50 (20%)	10/50 (20%)	25/50 (50%)	23/50 (46%)
Adjusted rate[b]	20.9%	22.3%	52.4%	61.8%
Terminal rate[c]	9/39 (23%)	8/32 (25%)	22/39 (56%)	15/23 (65%)
First incidence (days)	338	563	639	547
Poly-3 test[d]	P<0.001	P=0.535	P<0.001	P<0.001
Harderian Gland: Carcinoma				
Overall rate	1/50 (2%)	0/50 (0%)	3/50 (6%)	2/50 (4%)
Adjusted rate	2.1%	0.0%	6.4%	5.7%
Terminal rate	1/39 (3%)	0/32 (0%)	2/39 (5%)	1/23 (4%)
First incidence (days)	729 (T)	—[e]	711	711
Poly-3 test	P=0.115	P=0.512N	P=0.307	P=0.403
Harderian Gland: Adenoma or Carcinoma				
Overall rate	11/50 (22%)	10/50 (20%)	26/50 (52%)	25/50 (50%)
Adjusted rate	23.0%	22.3%	54.5%	67.0%
Terminal rate	10/39 (26%)	8/32 (25%)	23/39 (59%)	16/23 (70%)
First incidence (days)	338	563	639	547
Poly-3 test	P<0.001	P=0.568N	P<0.001	P<0.001
Liver: Hepatocellular Adenoma				
Overall rate	14/50 (28%)	14/50 (28%)	15/50 (30%)	10/50 (20%)
Adjusted rate	29.9%	31.3%	31.9%	27.5%
Terminal rate	14/39 (36%)	10/32 (31%)	14/39 (36%)	7/23 (30%)
First incidence (days)	729 (T)	640	716	429
Poly-3 test	P=0.451N	P=0.531	P=0.504	P=0.501N
Liver: Hepatocellular Carcinoma				
Overall rate	7/50 (14%)	3/50 (6%)	6/50 (12%)	2/50 (4%)
Adjusted rate	14.9%	6.8%	12.8%	5.7%
Terminal rate	6/39 (15%)	3/32 (9%)	5/39 (13%)	1/23 (4%)
First incidence (days)	673	729 (T)	715	680
Poly-3 test	P=0.253N	P=0.186N	P=0.501N	P=0.170N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	20/50 (40%)	15/50 (30%)	19/50 (38%)	10/50 (20%)
Adjusted rate	42.5%	33.5%	40.3%	27.5%
Terminal rate	19/39 (49%)	11/32 (34%)	17/39 (44%)	7/23 (30%)
First incidence (days)	673	640	715	429
Poly-3 test	P=0.183N	P=0.251N	P=0.500N	P=0.117N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	4/50 (8%)	1/50 (2%)	8/50 (16%)	5/50 (10%)
Adjusted rate	8.5%	2.3%	16.9%	14.1%
Terminal rate	3/39 (8%)	0/32 (0%)	5/39 (13%)	4/23 (17%)
First incidence (days)	701	633	639	667
Poly-3 test	P=0.062	P=0.196N	P=0.182	P=0.330
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	2/50 (4%)	5/50 (10%)	6/50 (12%)	4/50 (8%)
Adjusted rate	4.2%	11.2%	12.7%	11.3%
Terminal rate	0/39 (0%)	1/32 (3%)	5/39 (13%)	3/23 (13%)
First incidence (days)	677	647	645	680
Poly-3 test	P=0.203	P=0.196	P=0.134	P=0.218

Table B3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	6/50 (12%)	6/50 (12%)	14/50 (28%)	9/50 (18%)
Adjusted rate	12.7%	13.3%	29.3%	25.3%
Terminal rate	3/39 (8%)	1/32 (3%)	10/39 (26%)	7/23 (30%)
First incidence (days)	677	633	639	667
Poly-3 test	P=0.029	P=0.588	P=0.039	P=0.121
Ovary: Cystadenoma				
Overall rate	8/50 (16%)	4/50 (8%)	2/50 (4%)	3/50 (6%)
Adjusted rate	17.0%	9.1%	4.3%	8.5%
Terminal rate	7/39 (18%)	4/32 (13%)	2/39 (5%)	2/23 (9%)
First incidence (days)	659	729 (T)	729 (T)	704
Poly-3 test	P=0.112N	P=0.211N	P=0.046N	P=0.219N
Ovary: Benign Granulosa Cell Tumor				
Overall rate	0/50 (0%)	0/50 (0%)	3/50 (6%)	1/50 (2%)
Adjusted rate	0.0%	0.0%	6.4%	2.8%
Terminal rate	0/39 (0%)	0/32 (0%)	3/39 (8%)	1/23 (4%)
First incidence (days)	—	—	729 (T)	729 (T)
Poly-3 test	P=0.109	—[f]	P=0.119	P=0.444
Ovary: Benign or Malignant Granulosa Cell Tumor				
Overall rate	0/50 (0%)	0/50 (0%)	4/50 (8%)	1/50 (2%)
Adjusted rate	0.0%	0.0%	8.5%	2.8%
Terminal rate	0/39 (0%)	0/32 (0%)	4/39 (10%)	1/23 (4%)
First incidence (days)	—	—	729 (T)	729 (T)
Poly-3 test	P=0.090	—	P=0.061	P=0.444
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	2/48 (4%)	4/49 (8%)	4/48 (8%)	2/49 (4%)
Adjusted rate	4.5%	9.1%	8.8%	5.7%
Terminal rate	2/37 (5%)	2/32 (6%)	3/38 (8%)	1/23 (4%)
First incidence (days)	729 (T)	653	646	640
Poly-3 test	P=0.529	P=0.330	P=0.342	P=0.601
Skin (Subcutaneous Tissue): Fibrosarcoma				
Overall rate	2/50 (4%)	3/50 (6%)	3/50 (6%)	2/50 (4%)
Adjusted rate	4.2%	6.8%	6.3%	5.6%
Terminal rate	1/39 (3%)	1/32 (3%)	0/39 (0%)	0/23 (0%)
First incidence (days)	653	694	646	555
Poly-3 test	P=0.509	P=0.472	P=0.503	P=0.593
Skin (Subcutaneous Tissue): Fibrous Histiocytoma, Fibrosarcoma, or Sarcoma				
Overall rate	3/50 (6%)	5/50 (10%)	4/50 (8%)	3/50 (6%)
Adjusted rate	6.3%	11.2%	8.4%	8.3%
Terminal rate	1/39 (3%)	2/32 (6%)	1/39 (3%)	0/23 (0%)
First incidence (days)	653	640	646	555
Poly-3 test	P=0.539	P=0.324	P=0.500	P=0.533
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	3/50 (6%)	6/50 (12%)	24/50 (48%)	19/50 (38%)
Adjusted rate	6.4%	13.6%	51.0%	51.7%
Terminal rate	3/39 (8%)	5/32 (16%)	22/39 (56%)	14/23 (61%)
First incidence (days)	729 (T)	688	716	555
Poly-3 test	P<0.001	P=0.214	P<0.001	P<0.001

Table B3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Stomach (Forestomach): Squamous Cell Carcinoma				
Overall rate	0/50 (0%)	1/50 (2%)	1/50 (2%)	11/50 (22%)
Adjusted rate	0.0%	2.3%	2.1%	29.6%
Terminal rate	0/39 (0%)	0/32 (0%)	0/39 (0%)	3/23 (13%)
First incidence (days)	—	665	639	513
Poly-3 test	P<0.001	P=0.489	P=0.502	P<0.001
Stomach (Forestomach): Squamous Cell Papilloma or Carcinoma				
Overall rate	3/50 (6%)	7/50 (14%)	25/50 (50%)	29/50 (58%)
Adjusted rate	6.4%	15.7%	52.7%	75.4%
Terminal rate	3/39 (8%)	5/32 (16%)	22/39 (56%)	17/23 (74%)
First incidence (days)	729 (T)	665	639	513
Poly-3 test	P<0.001	P=0.136	P<0.001	P<0.001
Uterus: Stromal Polyp				
Overall rate	0/50 (0%)	1/50 (2%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	2.3%	6.4%	0.0%
Terminal rate	0/39 (0%)	0/32 (0%)	3/39 (8%)	0/23 (0%)
First incidence (days)	—	724	729 (T)	—
Poly-3 test	P=0.441	P=0.488	P=0.119	—
All Organs: Hemangioma				
Overall rate	0/50 (0%)	1/50 (2%)	1/50 (2%)	4/50 (8%)
Adjusted rate	0.0%	2.2%	2.1%	11.2%
Terminal rate	0/39 (0%)	0/32 (0%)	1/39 (3%)	1/23 (4%)
First incidence (days)	—	563	729 (T)	649
Poly-3 test	P=0.012	P=0.490	P=0.500	P=0.034
All Organs: Hemangiosarcoma				
Overall rate	4/50 (8%)	3/50 (6%)	2/50 (4%)	0/50 (0%)
Adjusted rate	8.5%	6.8%	4.3%	0.0%
Terminal rate	4/39 (10%)	3/32 (9%)	2/39 (5%)	0/23 (0%)
First incidence (days)	729 (T)	729 (T)	729 (T)	—
Poly-3 test	P=0.062N	P=0.534N	P=0.336N	P=0.107N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	4/50 (8%)	4/50 (8%)	3/50 (6%)	4/50 (8%)
Adjusted rate	8.5%	9.0%	6.4%	11.2%
Terminal rate	4/39 (10%)	3/32 (9%)	3/39 (8%)	1/23 (4%)
First incidence (days)	729 (T)	563	729 (T)	649
Poly-3 test	P=0.470	P=0.615	P=0.499N	P=0.490
All Organs: Malignant Lymphoma				
Overall rate	4/50 (8%)	5/50 (10%)	7/50 (14%)	6/50 (12%)
Adjusted rate	8.5%	11.0%	14.6%	16.4%
Terminal rate	4/39 (10%)	3/32 (9%)	6/39 (15%)	3/23 (13%)
First incidence (days)	729 (T)	354	190	429
Poly-3 test	P=0.156	P=0.481	P=0.275	P=0.227
All Organs: Benign Neoplasms				
Overall rate	29/50 (58%)	33/50 (66%)	45/50 (90%)	33/50 (66%)
Adjusted rate	60.2%	71.8%	93.9%	83.7%
Terminal rate	26/39 (67%)	25/32 (78%)	38/39 (97%)	20/23 (87%)
First incidence (days)	338	563	639	429
Poly-3 test	P<0.001	P=0.159	P<0.001	P=0.011

Table B3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
All Organs: Malignant Neoplasms				
Overall rate	24/50 (48%)	22/50 (44%)	30/50 (60%)	24/50 (48%)
Adjusted rate	49.1%	46.8%	60.1%	60.6%
Terminal rate	16/39 (41%)	12/32 (38%)	21/39 (54%)	10/23 (44%)
First incidence (days)	562	354	190	429
Poly-3 test	P=0.080	P=0.494N	P=0.185	P=0.192
All Organs: Benign or Malignant Neoplasms				
Overall rate	39/50 (78%)	41/50 (82%)	50/50 (100%)	40/50 (80%)
Adjusted rate	78.3%	86.1%	100.0%	97.6%
Terminal rate	30/39 (77%)	28/32 (88%)	39/39 (100%)	23/23 (100%)
First incidence (days)	338	354	190	429
Poly-3 test	P<0.001	P=0.226	P<0.001	P=0.005

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

Table B4a
Historical Incidence of Forestomach Neoplasms in Control Female B6C3F$_1$ Mice

Study	Incidence in Controls		
	Squamous Cell Papilloma	Squamous Cell Carcinoma	Squamous Cell Papilloma or Carcinoma
Historical Incidence in Controls Given NTP-2000 Diet[a]			
Acrylonitrile (gavage)	3/50	0/50	3/50
Citral (feed)	1/99	0/99	1/99
p,p′-Dichlorodiphenyl sulfone (feed)	0/50	0/50	0/50
Indium phosphide (inhalation)	0/50	1/50	1/50
60-Hz Magnetic fields (whole body exposure)	1/100	0/100	1/100
Methacrylonitrile (gavage)	0/50	0/50	0/50
o-Nitrotoluene (feed)	1/60	0/60	1/60
p-Nitrotoluene (feed)	0/50	0/50	0/50
Riddelliine (gavage)	0/50	0/50	0/50
Sodium nitrite (drinking water)	1/50	0/50	1/50
Vanadium pentoxide (inhalation)	2/50	0/50	2/50
Overall Historical Incidence in Controls Given NTP-2000 Diet			
Total (%)	9/659 (1.4%)	1/659 (0.2%)	10/659 (1.5%)
Mean ± standard deviation	1.4% ± 2.0%	0.2% ± 0.6%	1.6% ± 1.9%
Range	0%-6%	0%-2%	0%-6%
Historical Incidence in Water Gavage Controls Given NIH-07 Diet at Battelle Columbus Laboratories[b]			
Scopolamine hydrobromide trihydrate	0/51	0/51	0/51

[a] Data as of 20 December 2000
[b] Data as of 23 December 1999

Table B4b
Historical Incidence of Harderian Gland Neoplasms in Control Female B6C3F$_1$ Mice

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence in Controls Given NTP-2000 Diet[a]			
Acrylonitrile (gavage)	10/50	1/50	11/50
Citral (feed)	3/99	1/99	4/99
p,p′-Dichlorodiphenyl sulfone (feed)	3/50	0/50	3/50
Indium phosphide (inhalation)	3/50	0/50	3/50
60-Hz Magnetic fields (whole body exposure)	10/100	2/100	12/100
Methacrylonitrile (gavage)	2/50	2/50	4/50
o-Nitrotoluene (feed)	4/60	1/60	5/60
p-Nitrotoluene (feed)	2/50	0/50	2/50
Riddelliine (gavage)	2/50	2/50	4/50
Sodium nitrite (drinking water)	0/50	0/50	0/50
Vanadium pentoxide (inhalation)	2/50	0/50	2/50
Overall Historical Incidence in Controls Given NTP-2000 Diet			
Total (%)	41/659 (6.2%)	9/659 (1.4%)	50/659 (7.6%)
Mean ± standard deviation	6.2% ± 5.2%	1.3% ± 1.6%	7.5% ± 5.7%
Range	0%-20%	0%-4%	0%-22%
Historical Incidence in Water Gavage Controls Given NIH-07 Diet at Battelle Columbus Laboratories[b]			
Scopolamine hydrobromide trihydrate	1/51 (2.0%)	2/51 (4.0%)	3/51 (6.0%)

[a] Data as of 20 December 2000
[b] Data as of 23 December 1999

Table B4c
Historical Incidence of Ovarian Neoplasms in Control Female B6C3F$_1$ Mice

Study	Incidence in Controls			
	Hemangioma	Cystadenoma	Benign Granulosa Cell Tumor	Benign or Malignant Granulosa Cell Tumor
Historical Incidence in Controls Given NTP-2000 Diet[a]				
Acrylonitrile (gavage)	0/50	8/50	0/50	0/50
Citral (feed)	0/99	3/99	1/99	1/99
p,p′-Dichlorodiphenyl sulfone (feed)	0/50	2/50	1/50	1/50
Indium phosphide (inhalation)	0/47	0/47	0/47	0/47
60-Hz Magnetic fields (whole body exposure)	0/79	3/79	2/79	2/79
Methacrylonitrile (gavage)	2/50	2/50	0/50	0/50
o-Nitrotoluene (feed)	0/59	1/59	0/59	1/59
p-Nitrotoluene (feed)	1/48	3/48	1/48	1/48
Riddelliine (gavage)	0/49	1/49	0/49	0/49
Sodium nitrite (drinking water)	0/47	0/47	0/47	0/47
Vanadium pentoxide (inhalation)	0/48	1/48	0/48	0/48
Overall Historical Incidence in Controls Given NTP-2000 Diet				
Total (%)	3/626 (0.5%)	24/626 (3.8%)	5/626 (0.8%)	5/626 (0.8%)
Mean ± standard deviation	0.6% ± 1.3%	3.9% ± 4.4%	0.7% ± 1.0%	0.7% ± 1.0%
Range	0%-4%	0%-16%	0%-3%	0%-3%
Historical Incidence in Selected Controls Given NIH-07 Diet[b]				
Sodium nitrite (drinking water)	0/47	0/47	0/47	0/47
Diethanolamine (dermal)	3/49	2/49	0/49	0/49
Phenolphthalein (feed)	2/50	0/50	1/50	1/50
Scopolamine hydrobromide trihydrate (water gavage)	0/51	3/51	0/51	0/51
Overall Historical Incidence in Corn Oil Gavage Controls Given NIH-07 Diet[b]				
Total (%)	0/453	5/453 (1.1%)	5/453 (1.1%)	5/453 (1.1%)
Mean ± standard deviation		1.1% ± 1.4%	1.1% ± 1.5%	1.1% ± 1.5%
Range		0%-4%	0%-4%	0%-4%

[a] Data as of 20 December 2000
[b] Data as of 23 December 1999

Table B4d
Historical Incidence of Alveolar/bronchiolar Neoplasms in Control Female B6C3F$_1$ Mice

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence in Controls Given NTP-2000 Diet[a]			
Acrylonitrile (gavage)	4/50	2/50	6/50
Citral (feed)	5/99	6/99	11/99
p,p′-Dichlorodiphenyl sulfone (feed)	0/50	0/50	0/50
Indium phosphide (inhalation)	3/50	1/50	4/50
60-Hz Magnetic fields (whole body exposure)	9/95	2/95	11/95
Methacrylonitrile (gavage)	6/50	1/50	6/50
o-Nitrotoluene (feed)	2/60	3/60	5/60
p-Nitrotoluene (feed)	5/50	1/50	6/50
Riddelliine (gavage)	1/50	1/50	2/50
Sodium nitrite (drinking water)	1/50	0/50	1/50
Vanadium pentoxide (inhalation)	1/50	0/50	1/50
Overall Historical Incidence in Controls Given NTP-2000 Diet			
Total (%)	37/654 (5.7%)	17/654 (2.6%)	53/654 (8.1%)
Mean ± standard deviation	5.4% ± 4.0%	2.3% ± 2.0%	7.6% ± 4.7%
Range	0%-12%	0%-6%	0%-12%
Historical Incidence in Water Gavage Controls Given NIH-07 Diet at Battelle Columbus Laboratories[b]			
Scopolamine hydrobromide trihydrate	3/51 (6.0%)	1/51 (2.0%)	4/51 (8.0%)
Overall Historical Incidence in Feed Controls Given NIH-07 Diet[b]			
Total (%)	53/952 (5.6%)	31/952 (3.3%)	81/952 (8.5%)
Mean ± standard deviation	5.6% ± 2.6%	3.2% ± 3.1%	8.5% ± 3.6%
Range	2%-10%	0%-8%	2%-12%

[a] Data as of 20 December 2000
[b] Data as of 23 December 1999

Table B5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Acrylonitrile[a]

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death				1
Moribund	5	3	1	1
Natural deaths	6	15	10	25
Survivors				
Died last week of study				1
Terminal sacrifice	39	32	39	22
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(49)	(50)
Perforation				1 (2%)
Gallbladder	(50)	(50)	(49)	(50)
Infiltration cellular, lymphocyte				1 (2%)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Inflammation, suppurative		1 (2%)		
Peyer's patch, hyperplasia, lymphoid		1 (2%)	1 (2%)	
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	3 (6%)	9 (18%)	8 (16%)
Basophilic focus	1 (2%)	6 (12%)	4 (8%)	6 (12%)
Clear cell focus	5 (10%)	3 (6%)	6 (12%)	5 (10%)
Eosinophilic focus	9 (18%)	7 (14%)	18 (36%)	11 (22%)
Hematopoietic cell proliferation	1 (2%)	4 (8%)	3 (6%)	1 (2%)
Hyperplasia		1 (2%)		
Infarct			1 (2%)	
Inflammation, chronic active	42 (84%)	34 (68%)	40 (80%)	32 (64%)
Mineralization		1 (2%)		1 (2%)
Mixed cell focus	3 (6%)	15 (30%)	16 (32%)	13 (26%)
Necrosis	1 (2%)	3 (6%)	3 (6%)	6 (12%)
Pigmentation	6 (12%)	18 (36%)	13 (26%)	1 (2%)
Vacuolization cytoplasmic	9 (18%)	12 (24%)	15 (30%)	21 (42%)
Bile duct, cyst				1 (2%)
Centrilobular, degeneration	1 (2%)			
Portal, infiltration cellular, lymphocyte	2 (4%)			
Mesentery	(9)	(10)	(10)	(10)
Fibrosis	1 (11%)			
Fat, inflammation, chronic active	1 (11%)	1 (10%)		1 (10%)
Fat, necrosis	5 (56%)	7 (70%)	8 (80%)	6 (60%)
Pancreas	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)			
Infiltration cellular, mononuclear cell	2 (4%)	1 (2%)	9 (18%)	
Inflammation, chronic active		1 (2%)		
Acinus, atrophy	2 (4%)			
Salivary glands	(50)	(50)	(49)	(50)
Infiltration cellular, mononuclear cell	36 (72%)	32 (64%)	32 (65%)	15 (30%)

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

Table B5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Alimentary System (continued)				
Stomach, forestomach	(50)	(50)	(50)	(50)
Erosion, focal			1 (2%)	
Hyperkeratosis, diffuse	1 (2%)		2 (4%)	3 (6%)
Hyperkeratosis, focal	1 (2%)	1 (2%)		1 (2%)
Inflammation, chronic active, focal		1 (2%)	2 (4%)	1 (2%)
Inflammation, chronic active, multifocal	1 (2%)	1 (2%)		
Ulcer, multifocal	1 (2%)	1 (2%)		
Epithelium, hyperplasia, focal	2 (4%)	1 (2%)	5 (10%)	7 (14%)
Epithelium, hyperplasia, multifocal		1 (2%)		
Stomach, glandular	(50)	(50)	(50)	(50)
Hyperplasia, focal	1 (2%)		1 (2%)	
Inflammation, chronic active	1 (2%)			
Mineralization	2 (4%)		1 (2%)	
Glands, ectasia	11 (22%)	11 (22%)	6 (12%)	6 (12%)
Tooth			(1)	
Malformation			1 (100%)	
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Aorta, mineralization	1 (2%)	1 (2%)		1 (2%)
Heart	(50)	(50)	(50)	(50)
Inflammation, chronic active		1 (2%)	1 (2%)	3 (6%)
Mineralization	1 (2%)	1 (2%)		1 (2%)
Thrombosis			1 (2%)	
Valve, inflammation, suppurative		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	1 (2%)			
Cyst		1 (2%)		
Hematopoietic cell proliferation	1 (2%)	2 (4%)		
Hyperplasia	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Hypertrophy				1 (2%)
Inflammation, chronic active				1 (2%)
Metaplasia, osseous		1 (2%)		
Subcapsular, hyperplasia	50 (100%)	49 (98%)	47 (94%)	49 (98%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)			
Parathyroid gland	(43)	(39)	(37)	(34)
Cyst	1 (2%)	1 (3%)		
Pituitary gland	(48)	(49)	(48)	(49)
Pars distalis, angiectasis		1 (2%)	1 (2%)	
Pars distalis, cyst			1 (2%)	
Pars distalis, hyperplasia	10 (21%)	8 (16%)	6 (13%)	2 (4%)
Pars distalis, pigmentation		1 (2%)		
Thyroid gland	(50)	(50)	(49)	(50)
Infiltration cellular, lymphocyte		1 (2%)		
Follicle, cyst	6 (12%)	4 (8%)	3 (6%)	1 (2%)
Follicular cell, hyperplasia	1 (2%)			
General Body System				
None				

Table B5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Genital System				
Clitoral gland	(50)	(48)	(49)	(48)
Infiltration cellular, mononuclear cell	2 (4%)			
Inflammation, chronic active	3 (6%)	1 (2%)		1 (2%)
Pigmentation		1 (2%)		
Duct, cyst			1 (2%)	
Ovary	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	1 (2%)	2 (4%)	
Atrophy	6 (12%)	8 (16%)	45 (90%)	40 (80%)
Cyst	12 (24%)	20 (40%)	27 (54%)	19 (38%)
Mineralization			2 (4%)	2 (4%)
Pigmentation		1 (2%)	3 (6%)	2 (4%)
Thrombosis				2 (4%)
Uterus	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	2 (4%)	3 (6%)	1 (2%)
Cyst		1 (2%)		
Inflammation, suppurative	1 (2%)	2 (4%)		
Endometrium, hyperplasia, cystic	40 (80%)	41 (82%)	29 (58%)	19 (38%)
Lymphatic, angiectasis	1 (2%)			
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Myelofibrosis	1 (2%)	1 (2%)		1 (2%)
Myeloid cell, hyperplasia	1 (2%)	5 (10%)	3 (6%)	5 (10%)
Lymph node	(37)	(32)	(30)	(25)
Inguinal, hyperplasia, lymphoid			1 (3%)	
Mediastinal, hematopoietic cell proliferation		1 (3%)		
Mediastinal, hyperplasia, lymphoid	2 (5%)	4 (13%)	5 (17%)	4 (16%)
Renal, hematopoietic cell proliferation		1 (3%)		
Renal, hyperplasia, lymphoid		1 (3%)		
Lymph node, mandibular	(49)	(49)	(47)	(50)
Angiectasis				1 (2%)
Hematopoietic cell proliferation		1 (2%)		
Hyperplasia, lymphoid	4 (8%)	7 (14%)	12 (26%)	9 (18%)
Lymph node, mesenteric	(50)	(49)	(50)	(47)
Angiectasis		1 (2%)		
Ectasia	1 (2%)		2 (4%)	
Hematopoietic cell proliferation	1 (2%)	1 (2%)	1 (2%)	
Hyperplasia, lymphoid	3 (6%)	1 (2%)	3 (6%)	1 (2%)
Hyperplasia, plasma cell		1 (2%)		
Infiltration cellular, polymorphonuclear		1 (2%)	1 (2%)	
Inflammation, chronic active	1 (2%)	2 (4%)		
Spleen	(50)	(50)	(50)	(50)
Hematopoietic cell proliferation	17 (34%)	23 (46%)	24 (48%)	21 (42%)
Hyperplasia, lymphoid	18 (36%)	16 (32%)	17 (34%)	10 (20%)
Thymus	(50)	(50)	(47)	(50)
Atrophy	6 (12%)	11 (22%)	4 (9%)	10 (20%)
Ectopic parathyroid gland	1 (2%)	2 (4%)		
Hyperplasia, lymphoid	1 (2%)		2 (4%)	
Thymocyte, necrosis	1 (2%)			1 (2%)

Table B5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)		
Skin	(50)	(50)	(50)	(50)
Inflammation, chronic active		1 (2%)		1 (2%)
Ulcer		1 (2%)		
Epidermis, hyperplasia		1 (2%)		
Subcutaneous tissue, fibrosis	1 (2%)	2 (4%)	1 (2%)	
Subcutaneous tissue, hemorrhage		1 (2%)		
Subcutaneous tissue, infiltration cellular, mast cell		1 (2%)		
Subcutaneous tissue, inflammation, chronic active		2 (4%)	1 (2%)	
Musculoskeletal System				
Skeletal muscle	(3)	(3)	(2)	(1)
Inflammation, chronic active		1 (33%)		
Arteriole, mineralization		1 (33%)		
Nervous System				
None				
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Erythrophagocytosis	1 (2%)			1 (2%)
Fibrosis				1 (2%)
Hemorrhage			1 (2%)	
Inflammation, chronic active	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Inflammation, suppurative		2 (4%)		2 (4%)
Mineralization	1 (2%)	1 (2%)		
Necrosis			1 (2%)	
Pigmentation			1 (2%)	1 (2%)
Thrombosis		2 (4%)		
Alveolar epithelium, hyperplasia	2 (4%)	2 (4%)	4 (8%)	
Alveolus, infiltration cellular, histiocyte	2 (4%)	3 (6%)	4 (8%)	4 (8%)
Mediastinum, inflammation, chronic active		1 (2%)		
Nose	(50)	(50)	(50)	(50)
Nasolacrimal duct, inflammation, suppurative	1 (2%)			1 (2%)
Special Senses System				
Eye		(1)	(2)	(9)
Atrophy		1 (100%)	1 (50%)	7 (78%)
Synechia			1 (50%)	1 (11%)
Cornea, inflammation, chronic active		1 (100%)		5 (56%)
Lens, cataract				2 (22%)
Retina, degeneration			1 (50%)	
Harderian gland	(49)	(50)	(49)	(49)
Hyperplasia	5 (10%)	4 (8%)	6 (12%)	7 (14%)
Infiltration cellular, mononuclear cell	40 (82%)	30 (60%)	33 (67%)	23 (47%)
Bilateral, hyperplasia				1 (2%)

Table B5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Infarct	2 (4%)	3 (6%)		
Inflammation, chronic active	2 (4%)	2 (4%)		
Metaplasia, osseous	2 (4%)	1 (2%)		1 (2%)
Mineralization	4 (8%)	3 (6%)	2 (4%)	4 (8%)
Nephropathy	14 (28%)	6 (12%)	10 (20%)	5 (10%)
Cortex, cyst		2 (4%)	2 (4%)	1 (2%)
Cortex, hydronephrosis			1 (2%)	
Medulla, cyst	1 (2%)			
Renal tubule, hyperplasia		1 (2%)		
Renal tubule, vacuolization cytoplasmic	1 (2%)			

APPENDIX C
GENETIC TOXICOLOGY

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GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocol

Testing was performed as reported by Zeiger and Haworth (1985). Acrylonitrile was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA97, TA98, TA100, and TA1535 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of acrylonitrile. In the absence of toxicity, 10,000 µg/plate was selected as the high dose. Positive trials were repeated under the conditions that elicited the positive response with the same or greater S9 fraction.

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

Mouse Lymphoma Mutagenicity Test Protocol

The experimental protocol is presented in detail by Myhr *et al.* (1985). Acrylonitrile was supplied as a coded aliquot by Radian Corporation. The high dose of 50 nL/mL acrylonitrile was determined by toxicity. L5178Y mouse lymphoma cells were maintained at 37° C as suspension cultures in supplemented Fischer's medium; normal cycling time was approximately 10 hours. To reduce the number of spontaneously occurring cells resistant to trifluorothymidine (TFT), subcultures were exposed to medium containing thymidine, hypoxanthine, methotrexate, and glycine for 1 day; to medium containing thymidine, hypoxanthine, and glycine for 1 day; and to normal medium for 3 to 5 days. For cloning, the horse serum content was increased and Noble agar was added.

The range of treatment levels within the experiment, including concurrent positive and solvent controls, was generally replicated in a second trial. Treated cultures contained 6×10^6 cells in 10 mL medium. Incubation with acrylonitrile continued for 4 hours, at which time the medium plus acrylonitrile was removed, and the cells were resuspended in fresh medium and incubated for an additional 2 days to express the mutant phenotype. Cell density was monitored so that log phase growth was maintained. After the 48-hour expression period, cells were plated in medium and soft agar supplemented with TFT for selection of TFT-resistant cells, and cells were plated in nonselective medium and soft agar to determine cloning efficiency. Plates were incubated at 37° C in 5% CO_2 for 10 to 12 days. The test was initially performed without S9. Because a clearly positive response was obtained, the test was not conducted with S9.

Minimum criteria for accepting an experiment as valid and a detailed description of the statistical analysis and data evaluation are presented by Caspary *et al.* (1988). All data were evaluated statistically for trend and peak responses. Both responses had to be significant ($P \leq 0.05$) for acrylonitrile to be considered positive, i.e., capable of inducing TFT resistance. A single significant response led to a call of "questionable," and the absence of both a trend and peak response resulted in a "negative" call.

Chinese Hamster Ovary Cell Cytogenetics Protocols

Testing was performed as reported by Gulati *et al.* (1985). Acrylonitrile was sent to the laboratory as a coded aliquot by Radian Corporation. It was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least three doses of acrylonitrile; the high dose was limited by toxicity. A single flask per dose was used, and tests yielding equivocal or positive results were repeated.

Sister Chromatid Exchange Test: In the SCE test without S9, CHO cells were incubated for 26 to 28 hours with acrylonitrile in supplemented McCoy's 5A medium. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 26 hours, the medium containing acrylonitrile was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with acrylonitrile, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no acrylonitrile. Incubation proceeded for an additional 26 to 28 hours, with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9. All slides were scored blind, and those from a single test were read by the same person. Fifty second-division metaphase cells were scored for frequency of SCEs/cell from each dose level. Because significant acrylonitrile-induced cell cycle delay was seen, total incubation time was lengthened to 40 hours to ensure a sufficient number of scorable (second-division metaphase) cells.

Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points (Galloway *et al.,* 1987). An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. An increase of 20% or greater at any single dose was considered weak evidence of activity; increases at two or more doses resulted in a determination that the trial was positive. A statistically significant trend ($P<0.005$) in the absence of any responses reaching 20% above background led to a call of equivocal.

Chromosomal Aberrations Test: In the Abs test without S9, cells were incubated in McCoy's 5A medium with acrylonitrile for 10 to 10.5 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with acrylonitrile and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 10 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind, and those from a single test were read by the same person. One hundred first-division metaphase cells were scored at each dose level. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

Chromosomal aberration data are presented as percentage of cells with aberrations. To arrive at a statistical call for a trial, analyses were conducted on both the dose response curve and individual dose points. For a single trial, a statistically significant ($P\leq0.05$) difference for one dose point and a significant trend ($P\leq0.015$) were considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was positive. A positive trend test in the absence of a statistically significant increase at any one dose resulted in an equivocal call (Galloway *et al.,* 1987). Ultimately, the trial calls were based on a consideration of the statistical analyses as well as the biological information available to the reviewers.

Drosophila melanogaster Test Protocols

The assays for induction of sex-linked recessive lethal (SLRL) mutations and chromosomal reciprocal translocations (RTs) were performed with adult flies as described by Valencia *et al.* (1985) and Foureman *et al.* (1994). Acrylonitrile was supplied as a coded aliquot by Radian Corporation.

Sex-Linked Recessive Lethal Mutation Test: Acrylonitrile was assayed in the SLRL test by feeding for 3 days to adult Canton-S wild-type males no more than 24 hours old at the beginning of treatment. Because no response was obtained, acrylonitrile was retested by injection into adult males.

To administer acrylonitrile by injection, a glass Pasteur pipette was drawn out in a flame to a microfine filament, and the tip was broken off to allow delivery of the test solution. Injection was performed either manually, by attaching a rubber bulb to the other end of the pipette and forcing through sufficient solution (0.2 to 0.3 µL) to slightly distend the abdomen of the fly, or by attaching the pipette to a microinjector that automatically delivered a calibrated volume. Flies were anesthetized with ether and immobilized on a strip of tape. Injection into the thorax, under the wing, was performed with the aid of a dissecting microscope.

Toxicity tests were performed to set concentrations of acrylonitrile at a level that would induce 30% mortality after 72 hours of feeding or 24 hours after injection, while keeping induced sterility at an acceptable level. Canton-S males were allowed to feed for 72 hours on a solution of acrylonitrile in 5% sucrose. In the injection experiments, 24- to 72-hour old Canton-S males were treated with a solution of acrylonitrile dissolved in saline and allowed to recover for 24 hours. A concurrent saline control group was also included. Treated males were mated to three *Basc* females for 3 days and were given fresh females at 2-day intervals to produce three matings of 3, 2, and 2 days (in each case, sample sperm from successive matings was 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. One cluster of 4 lethals was identified and removed from the injection control group. 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 *et al.,* 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 tested 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 or equal to 0.10 or if the frequency in the treatment group was less than 0.10%.

Reciprocal Translocation Test: Acrylonitrile was assayed for induction of reciprocal translocations using the injection route. The treatment regimen was essentially the same as that for the SLRL test, except that Canton-S males were mated *en masse* to marker (*bw;st or bw;e*) females. The females were transferred to fresh medium every 3 to 4 days for a period of about 3 weeks to produce a total of six broods. The results of the SLRL test were used to determine the germ cell stages most likely to be affected by acrylonitrile. F_1 heterozygous males were backcrossed individually to *bw;st* females, and the F_2 progeny were screened for pseudolinkage, which results from the induction of a translocation in a germ cell of the parental male. Flies suspected of carrying reciprocal translocations were retested to confirm the findings. The translocation data were compared to the concurrent and historical controls, and significance was analyzed according to the conditional binomial response of Kastenbaum and Bowman (1970).

Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 14-week toxicity study, peripheral blood samples were obtained from surviving male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs) in up to 10 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 NCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dosed group is less than or equal to 0.025 divided by the number of dosed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Results of the 14-week studies were accepted without repeat tests, because additional test data could not be obtained. Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

Evaluation Protocol

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

Results

Acrylonitrile (100 to 10,000 µg/plate) was mutagenic in *S. typhimurium* strain TA100 in the presence of hamster liver S9 enzymes (Table C1; Zeiger and Haworth, 1985); it was also mutagenic in strain TA1535 with rat and hamster S9. It was not mutagenic in strain TA97 or TA98, with or without S9. Acrylonitrile induced mutations in mouse lymphoma L5178Y cells at concentrations of 12.5 nL/mL and higher in the absence of S9 (Table C2; Myhr *et al.,* 1985). In CHO cells, acrylonitrile induced SCEs with and without S9 (Table C3; Gulati *et al.,* 1985); Abs were significantly increased by acrylonitrile only in the presence of S9 (Table C4; Gulati *et al.,* 1985). No induction of sex-linked recessive lethal mutations in germ cells of male *D. melanogaster* was observed when 415 ppm acrylonitrile was administered in feed with sucrose or 3,475 ppm in saline was administered by injection (Table C5; Foureman *et al.,* 1994). Despite the results of the statistical analysis of the data ($P<0.001$), the frequency of lethal mutations in flies treated by injection (0.10%) was insufficient for a positive call. The control frequency in the injection test was unusually low and comparison of this extreme value with the low level of lethals observed in the treated flies produced the low P value in the injection experiment. Results of the reciprocal translocation test were also negative (Table C6).

In contrast to the induction of chromosomal damage in mammalian cells *in vitro,* no increase in the frequency of micronucleated NCEs was observed in peripheral blood samples from male or female mice administered acrylonitrile by gavage for 14 weeks (Table C7).

Acrylonitrile induced genetic damage *in vitro* in bacterial and mammalian cells, but *in vivo* tests in *D. melanogaster* and mice were negative.

Table C1
Mutagenicity of Acrylonitrile in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] -S9	+hamster S9 5%	+hamster S9 10%	+hamster S9 10%	+hamster S9 30%
TA100	0	99 ± 5.6	94 ± 1.5	92 ± 6.7	95 ± 2.1	115 ± 9.3
	100	96 ± 4.9	89 ± 7.4	106 ± 1.5	98 ± 7.1	126 ± 7.2
	333	89 ± 0.9	75 ± 8.7	131 ± 8.1	100 ± 6.3	161 ± 4.2
	1,000	71 ± 4.7	102 ± 7.8	143 ± 1.8	122 ± 12.5	260 ± 9.1
	3,333	60 ± 6.9	100 ± 5.5	164 ± 1.2	144 ± 7.3	373 ± 8.1
	6,666		101 ± 4.3		165 ± 7.6	435 ± 8.2
	6,667			178 ± 15.9[c]		
	10,000	61 ± 4.4[c]				
Trial summary		Negative	Negative	Weakly Positive	Weakly Positive	Positive
Positive control[d]		1,188 ± 67.7	3,138 ± 158.3	917 ± 34.0	1,197 ± 90.7	1,115 ± 18.3

Strain	Dose (µg/plate)	+rat S9 5%	+rat S9 10%	+rat S9 10%	+rat S9 30%
TA100 (continued)	0	90 ± 10.2	92 ± 6.9	93 ± 4.6	93 ± 3.1
	100	97 ± 6.0	88 ± 3.4	100 ± 4.3	100 ± 5.7
	333	95 ± 7.1	99 ± 6.6	104 ± 2.6	109 ± 4.4
	1,000	94 ± 2.4	90 ± 8.4	106 ± 6.9	125 ± 2.0
	3,333	96 ± 6.0	95 ± 0.9	109 ± 9.2	140 ± 1.5
	6,666	108 ± 3.8		104 ± 7.0	124 ± 5.1
	6,667		71 ± 5.9[c]		
Trial summary		Negative	Negative	Negative	Equivocal
Positive control		2,871 ± 62.8	1,292 ± 62.5	1,523 ± 24.1	509 ± 5.6

Strain	Dose (µg/plate)	-S9	5%	10%	10%	30%
TA1535	0	25 ± 1.0	14 ± 1.2	16 ± 3.5	11 ± 2.3	23 ± 4.2
	100	25 ± 1.8	13 ± 1.2	23 ± 3.7	19 ± 2.6	33 ± 2.1
	333	23 ± 0.6	18 ± 3.2	33 ± 2.5	30 ± 2.1	67 ± 4.2
	1,000	18 ± 0.3	24 ± 2.1	79 ± 5.2	53 ± 3.2	161 ± 7.2
	3,333	10 ± 2.5	36 ± 3.5	94 ± 3.8	95 ± 5.2	364 ± 19.7
	6,666		43 ± 7.5[c]		105 ± 3.9	432 ± 13.0
	6,667			85 ± 9.2[c]		
	10,000	6 ± 0.6[c]				
Trial summary		Negative	Positive	Positive	Positive	Positive
Positive control		829 ± 35.5	161 ± 5.9	123 ± 11.6	118 ± 9.0	229 ± 14.4

Strain	Dose (µg/plate)	+rat S9 5%	+rat S9 10%	+rat S9 10%	+rat S9 30%
TA1535 (continued)	0	12 ± 3.4	11 ± 3.1	12 ± 3.5	19 ± 1.2
	100	15 ± 0.9	14 ± 1.5	14 ± 3.1	19 ± 1.2
	333	16 ± 3.2	21 ± 1.5	23 ± 4.4	32 ± 1.9
	1,000	25 ± 2.1	27 ± 7.0	23 ± 1.2	51 ± 3.8
	3,333	27 ± 2.7	24 ± 2.6	47 ± 6.4	98 ± 8.6
	6,666	23 ± 1.5		46 ± 3.2[c]	107 ± 3.5
	6,667		21 ± 3.3[c]		
Trial summary		Weakly Positive	Weakly Positive	Positive	Positive
Positive control		173 ± 10.7	121 ± 2.5	134 ± 2.7	137 ± 7.2

Table C1
Mutagenicity of Acrylonitrile in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate -S9	Revertants/Plate + S9 10% hamster	Revertants/Plate + S9 10% rat
TA97	0	65 ± 3.3	102 ± 1.8	124 ± 8.0
	100	82 ± 3.5	101 ± 4.0	120 ± 8.3
	333	81 ± 6.5	104 ± 1.7	110 ± 8.4
	1,000	59 ± 8.5	112 ± 8.9	111 ± 14.6
	3,333	51 ± 9.0	96 ± 11.3	95 ± 6.0
	6,666			
	6,667		83 ± 3.5[c]	100 ± 10.9[c]
	10,000	25 ± 1.5[c]		
Trial summary		Negative	Negative	Negative
Positive control		583 ± 103.1	476 ± 36.2	578 ± 15.9
TA98	0	15 ± 0.3	33 ± 2.7	30 ± 2.5
	100	16 ± 3.5	27 ± 1.2	26 ± 2.7
	333	18 ± 0.9	24 ± 1.2	28 ± 3.9
	1,000	17 ± 3.8	24 ± 1.5	20 ± 4.1
	3,333	15 ± 0.7	27 ± 7.4	27 ± 5.2
	6,666			
	6,667		11 ± 2.6[c]	24 ± 0.6[c]
	10,000	6 ± 1.5[c]		
Trial summary		Negative	Negative	Negative
Positive control		1,374 ± 46.6	846 ± 19.8	1,128 ± 54.0

[a] Studies were performed at Microbiological Associates, Inc. The detailed protocol and these data are presented by Zeiger and Haworth (1985). 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 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

Table C2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by Acrylonitrile[a]

Compound	Concentration	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction[b]	Average Mutant Fraction
-S9						
Trial 1						
Ethanol (nL/mL)[c]		85	105	58	23	
		69	92	91	44	
		72	107	55	25	
		77	96	88	38	33
Acrylonitrile (nL/mL)	3.13	62	69	53	28	
		79	53	78	33	
		67	53	64	32	31
	6.25	52	68	65	41	
		58	71	61	35	
		70	63	74	35	37
	12.5	71	47	186	88	
		86	71	138	53	71*
	25	40	10	395	333	
		72	31	337	156	
		79	31	346	147	212*
	50	46	3	588	428	
		50	3	500	336	
		34	3	491	479	414*
	100	Lethal				
Ethylmethane sulfonate (µg/mL)[d]	250	54	50	682	421	
		49	42	686	464	
		49	50	684	462	449*

Table C2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by Acrylonitrile

Compound	Concentration	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
-S9						
Trial 2						
Ethanol (nL/mL)		68	83	90	44	
		97	120	83	29	
		78	97	69	30	34
Acrylonitrile (nL/mL)	5	69	66	45	22	
		45	51	35	26	24
	10	64	69	59	31	
		84	71	52	21	
		52	61	27	17	23
	20	64	56	95	49	
		55	57	69	42	
		83	51	142	57	49
	30	78	46	155	67	
		99	46	226	76	
		74	41	164	74	72*
	40	68	29	226	111	
		74	24	381	173	142*
	50	88	22	407	155	
		53	7	365	230	192*
Ethylmethane sulfonate (μg/mL)	250	59	37	820	465	
Methylmethane sulfonate (μg/mL)[d]	10	26	6	447	562	

* Positive response ($P \leq 0.05$) versus the solvent control
[a] Study was performed at Litton Bionetics, Inc. The detailed protocol and these data are presented by Myhr *et al.* (1985).
[b] Mutant fraction=mutant cells/10^6 clonable cells
[c] Solvent control
[d] Positive control

TABLE C3
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Acrylonitrile[a]

Compound	Dose (µg/mL)	Total Cells Scored	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome[b] (%)
-S9								
Trial 1								
Summary: Positive								
Dimethylsulfoxide[c]		50	1,049	417	0.39	8.3	26.0	
Acrylonitrile	0.16	50	1,039	397	0.38	7.9	26.0	-3.88
	0.5	50	1,036	409	0.39	8.2	26.0	-0.69
	1.6	50	1,046	418	0.39	8.4	26.0	0.53
	5	50	1,052	450	0.42	9.0	26.0	7.60
	16	50	1,045	504	0.48	10.1	26.0	21.32*
	50	Lethal						
					P<0.001[d]			
Mitomycin-C[e]	0.01	50	1,047	2,332	2.22	46.6	26.0	460.30
Trial 2								
Summary: Positive								
Dimethylsulfoxide		50	1,036	410	0.39	8.2	26.0	
Acrylonitrile	10	50	1,046	535	0.51	10.7	28.0	29.24*
	20	50	1,044	614	0.58	12.3	28.0	48.61*
	20	50	1,049	686	0.65	13.7	38.0[f]	65.24*
	30	Lethal					28.0	
	30	50	1,046	980	0.93	19.6	38.0[f]	136.74*
	40	Lethal					38.0[f]	
					P<0.001			
Mitomycin-C	0.01	50	1,040	2,322	2.23	46.4	26.0	464.17

TABLE C3
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Acrylonitrile

Compound	Dose (µg/mL)	Total Cells Scored	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome (%)
+S9								
Trial 1								
Summary: Positive								
Dimethylsulfoxide		50	1,043	421	0.40	8.4	26.0	
Acrylonitrile	1.6	50	1,047	425	0.40	8.5	26.0	0.56
	5	50	1,049	416	0.39	8.3	26.0	-1.76
	16	50	1,049	411	0.39	8.2	26.0	-2.93
	50	50	1,041	544	0.52	10.9	26.0	29.46*
	160	Lethal					26.0	
					P<0.001			
Cyclophosphamide[e]	1.5	50	1,042	1,361	1.30	27.2	26.0	223.59
Trial 2								
Summary: Positive								
Dimethylsulfoxide		50	1,041	407	0.39	8.1	26.0	
Acrylonitrile	10	50	1,036	438	0.42	8.8	28.0	8.13
	25	50	1,038	513	0.49	10.3	28.0	26.41*
	50	50	1,027	620	0.60	12.4	28.0	54.41*
	75	50	1,047	850	0.81	17.0	28.0	107.65*
	75	50	1,046	788	0.75	15.8	38.0[f]	92.68*
	150	50	1,040	1,063	1.02	21.3	38.0[f]	161.43*
					P<0.001			
Cyclophosphamide	2	50	1,050	1,929	1.83	38.6	26.0	369.89

* Positive response (≥20% increase over solvent control)
[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol and these data are presented by Gulati *et al.* (1985). SCE=sister chromatid exchange; BrdU=bromodeoxyuridine
[b] SCEs/chromosome in treated cells versus SCEs/chromosome in solvent control cells
[c] Solvent control
[d] Significance of SCEs/chromosome tested by the linear regression trend test versus log of the dose
[e] Positive control
[f] Due to cell cycle delay, harvest time was extended to maximize the number of second-division metaphase cells available for analysis.

Table C4
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Acrylonitrile[a]

Compound	Dose (µg/mL)	Total Cells Scored	Number of Aberrations	Aberrations/ Cell	Cells with Aberrations (%)
-S9					
Trial 1					
Harvest time: 12.5 hours					
Summary: Negative					
Dimethylsulfoxide[b]		100	4	0.04	4.0
Acrylonitrile	5	100	1	0.01	1.0
	10	100	1	0.01	1.0
	25	100	3	0.03	3.0
	50	100	2	0.02	2.0
					P=0.650[c]
Mitomycin-C[d]	0.5	100	99	0.99	63.0
Trial 2					
Harvest time: 12.0 hours					
Summary: Negative					
Dimethylsulfoxide		100	0	0.00	0.0
Acrylonitrile	5	100	2	0.02	2.0
	10	100	2	0.02	2.0
	25	100	1	0.01	1.0
	50	100	1	0.01	1.0
	75	Lethal			
					P=0.398
Mitomycin-C	0.25	100	53	0.53	38.0

Table C4
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Acrylonitrile

Compound	Dose (µg/mL)	Total Cells Scored	Number of Aberrations	Aberrations/ Cell	Cells with Aberrations (%)
+S9					
Trial 1					
Harvest time: 12.0 hours					
Summary: Weakly Positive					
Dimethylsulfoxide		100	0	0.00	0.0
Acrylonitrile	1	100	0	0.00	0.0
	5	100	0	0.00	0.0
	10	100	3	0.03	3.0
	25	100	2	0.02	2.0
	50	100	2	0.02	2.0
	100	100	6	0.06	6.0*
	200	Lethal			
					P<0.001
Cyclophosphamide[d]	50	100	74	0.74	51.0
Trial 2					
Harvest time: 12.0 hours					
Summary: Weakly Positive					
Dimethylsulfoxide		100	2	0.02	2.0
Acrylonitrile	25	100	4	0.04	4.0
	50	100	5	0.05	4.0
	75	100	5	0.05	5.0
	100	100	13	0.13	11.0*
	150	Lethal			
					P=0.006
Cyclophosphamide	25	100	49	0.49	32.0

* Positive response ($P \leq 0.05$) versus the solvent control
[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol and these data are presented by Gulati *et al.* (1985).
[b] Solvent control
[c] Significance of percent cells with aberrations tested by the linear regression trend test versus log of the dose
[d] Positive control

TABLE C5
Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by Acrylonitrile[a]

Route of Exposure	Dose (ppm)	Incidence of Death (%)	Incidence of Sterility (%)	No. of Lethals/No. of X Chromosomes Tested			Total[b]
				Mating 1	Mating 2	Mating 3	
Feeding	415	2	0	1/2,226	1/2,328	0/1,000	2/5,554 (0.04%)
	0			0/1,955	2/1,998	1/1,356	3/5,309 (0.06%)
							P=0.523[c]
Injection	3,475	49	6	2/1,754	0/1,755	3/1,606	5/5,115 (0.10%)
	0			0/1,920	0/1,879	0/1,899	0/5,698 (0.00%)
							P<0.001

[a] Study was performed at Brown University. The detailed protocol and these data are presented by Foureman *et al.* (1994). The mean mutant frequency from 518 negative control experiments is 0.074% (Mason *et al.,* 1992).
[b] Total number of lethal mutations/total number of X chromosomes tested for three mating trials
[c] Significance of total number of lethal mutations/total number of X chromosomes tested by a normal approximation to the binomial test (Margolin *et al.,* 1983).

TABLE C6
Induction of Reciprocal Translocations in *Drosophila melanogaster* by Acrylonitrile[a]

Route of Exposure	Dose (ppm)	Translocations/Total F_1 Tested						No. of Tests	Total No. of Translocations	Total Translocations (%)
		1	2	3	4	5	6			
Injection	3,280	1/1,740	0/2,088	0/2,156	0/169	0/57	0	6,210	1	0.02
Concurrent control								42,695	0	0.00
Historical control								116,163	2	0.00
										P=0.057[b]

[a] Study was performed at Brown University. The detailed protocol is presented by Foureman *et al*. (1994).
[b] Significance of percent translocations tested by the conditional binomial response test (Kastenbaum and Bowman, 1970)

Table C7
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with Acrylonitrile by Gavage for 14 Weeks[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]
Male				
Water[d]		9	0.89 ± 0.29	
Acrylonitrile	5	10	0.70 ± 0.15	0.7436
	10	10	0.95 ± 0.22	0.4223
	20	10	0.55 ± 0.20	0.8921
	40	1	Toxic	
			P=0.848[e]	
Female				
Water		10	0.50 ± 0.17	
Acrylonitrile	5	10	0.80 ± 0.20	0.1196
	10	10	1.00 ± 0.24	0.0339
	20	10	0.70 ± 0.19	0.2070
	40	7	1.07 ± 0.17	0.0279
			P=0.085	

[a] Study was performed at SITEK Research Laboratories. The detailed protocol is presented by MacGregor *et al.* (1990).
[b] NCE=normochromatic erythrocyte
[c] Mean ± standard error
[d] Pairwise comparison with the vehicle control; significant at P≤0.008 (males) or P≤0.006 (females) (ILS, 1990)
[e] Vehicle control
Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

APPENDIX D
CLINICAL PATHOLOGY RESULTS

TABLE D1 Hematology Data for Mice in the 14-Week Gavage Study of Acrylonitrile 170

Table D1
Hematology Data for Mice in the 14-Week Gavage Study of Acrylonitrile[a]

	Vehicle Control	5 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg	60 mg/kg
Male						
n	9	10	9	10	1	0[b]
Hematocrit (%)	51.4 ± 0.7	50.6 ± 0.5	50.5 ± 1.1	51.2 ± 0.9	52.2	
Hemoglobin (g/dL)	16.6 ± 0.3	16.4 ± 0.2	16.2 ± 0.3	16.6 ± 0.4	16.9	
Erythrocytes (10^6/μL)	10.69 ± 0.18	10.54 ± 0.11	10.44 ± 0.22	10.56 ± 0.20	10.10	
Reticulocytes (10^6/μL)	0.13 ± 0.02	0.11 ± 0.02	0.12 ± 0.01	0.14 ± 0.01	0.18	
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	
Mean cell volume (fL)	48.1 ± 0.3	48.0 ± 0.2	48.4 ± 0.2	48.5 ± 0.2	51.6	
Mean cell hemoglobin (pg)	15.5 ± 0.1	15.6 ± 0.1	15.5 ± 0.1	15.7 ± 0.1	16.7	
Mean cell hemoglobin concentration (g/dL)	32.3 ± 0.2	32.4 ± 0.3	32.1 ± 0.2	32.4 ± 0.3	32.3	
Platelets (10^3/μL)	808.8 ± 38.9	757.8 ± 32.3	730.8 ± 54.4	646.1 ± 40.1*	693.0	
Leukocytes (10^3/μL)	4.71 ± 0.20	5.00 ± 0.51	4.37 ± 0.30	3.28 ± 0.40*	1.80	
Segmented neutrophils (10^3/μL)	0.64 ± 0.09	0.66 ± 0.09	0.50 ± 0.06	0.46 ± 0.13	0.20	
Bands (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	
Lymphocytes (10^3/μL)	3.95 ± 0.20	4.24 ± 0.44	3.83 ± 0.26	2.78 ± 0.28*	1.57	
Monocytes (10^3/μL)	0.09 ± 0.02	0.02 ± 0.01*	0.03 ± 0.02	0.03 ± 0.01	0.04	
Basophils (10^3/μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000	
Eosinophils (10^3/μL)	0.03 ± 0.01	0.09 ± 0.03	0.01 ± 0.01	0.01 ± 0.01	0.00	
Female						
n	10	10	10	10	7	0
Hematocrit (%)	50.5 ± 0.8	48.6 ± 0.7	49.0 ± 0.6	49.1 ± 0.5	47.5 ± 0.8*	
Hemoglobin (g/dL)	16.6 ± 0.2	15.9 ± 0.2*	15.9 ± 0.2*	15.9 ± 0.1**	15.0 ± 0.3**	
Erythrocytes (10^6/μL)	10.45 ± 0.18	10.05 ± 0.16	10.06 ± 0.13	10.07 ± 0.09	9.40 ± 0.16**	
Reticulocytes (10^6/μL)	0.10 ± 0.01	0.10 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.11 ± 0.01	
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
Mean cell volume (fL)	48.3 ± 0.2	48.4 ± 0.1	48.7 ± 0.2*	48.8 ± 0.2	50.5 ± 0.2**	
Mean cell hemoglobin (pg)	15.9 ± 0.1	15.9 ± 0.1	15.8 ± 0.1	15.8 ± 0.1	15.9 ± 0.1	
Mean cell hemoglobin concentration (g/dL)	33.0 ± 0.2	32.8 ± 0.2	32.5 ± 0.2	32.4 ± 0.2*	31.5 ± 0.1**	
Platelets (10^3/μL)	655.4 ± 45.6	721.8 ± 43.9	647.2 ± 37.0	664.0 ± 42.4	780.0 ± 57.7	
Leukocytes (10^3/μL)	3.60 ± 0.21	3.50 ± 0.20	3.62 ± 0.41	3.28 ± 0.41	2.23 ± 0.26**	
Segmented neutrophils (10^3/μL)	0.51 ± 0.05	0.49 ± 0.06	0.63 ± 0.14	0.31 ± 0.04	0.32 ± 0.11	
Bands (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
Lymphocytes (10^3/μL)	3.02 ± 0.19	2.95 ± 0.17	2.93 ± 0.28	2.93 ± 0.37	1.89 ± 0.16**	
Monocytes (10^3/μL)	0.04 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	
Basophils (10^3/μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	
Eosinophils (10^3/μL)	0.03 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] No data were available for the 60 mg/kg groups due to 100% mortality.

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

TABLE E1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Week Gavage Study of Acrylonitrile 172

Table E1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Week Gavage Study of Acrylonitrile[a]

	Vehicle Control	5 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg	60 mg/kg
Male						
n	9	10	10	10	1	0[b]
Necropsy body wt	35.0 ± 1.7	34.2 ± 0.6	34.8 ± 0.8	32.2 ± 0.8	34.7	
Heart						
Absolute	0.155 ± 0.005	0.160 ± 0.007	0.172 ± 0.007	0.186 ± 0.007**	0.197	
Relative	4.486 ± 0.177	4.685 ± 0.214	4.956 ± 0.197	5.817 ± 0.299**	5.677	
R. Kidney						
Absolute	0.257 ± 0.008	0.254 ± 0.006	0.258 ± 0.004	0.249 ± 0.005	0.263	
Relative	7.436 ± 0.253	7.427 ± 0.142	7.445 ± 0.175	7.736 ± 0.188	7.579	
Liver						
Absolute	1.462 ± 0.074	1.432 ± 0.018	1.496 ± 0.049	1.397 ± 0.036	1.686	
Relative	41.790 ± 0.650	41.931 ± 0.453	43.004 ± 1.154	43.378 ± 0.846	48.588	
Lung						
Absolute	0.319 ± 0.011	0.309 ± 0.011[c]	0.295 ± 0.010	0.312 ± 0.011	0.308	
Relative	9.276 ± 0.477	9.005 ± 0.246[c]	8.504 ± 0.294	9.700 ± 0.319	8.876	
Spleen						
Absolute	0.063 ± 0.003	0.063 ± 0.002	0.065 ± 0.002	0.061 ± 0.001	0.067	
Relative	1.809 ± 0.059	1.853 ± 0.053	1.874 ± 0.099	1.889 ± 0.031	1.931	
R. Testis						
Absolute	0.112 ± 0.002	0.115 ± 0.002	0.116 ± 0.002	0.113 ± 0.002	0.121	
Relative	3.280 ± 0.186	3.368 ± 0.058	3.345 ± 0.086	3.528 ± 0.076	3.487	
Thymus						
Absolute	0.044 ± 0.005	0.044 ± 0.003	0.044 ± 0.004	0.046 ± 0.004	0.041	
Relative	1.241 ± 0.106	1.289 ± 0.123	1.265 ± 0.096	1.424 ± 0.107	1.182	

Table E1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Week Gavage Study of Acrylonitrile

	Vehicle Control	5 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg	60 mg/kg
Female						
n	10	10	10	10	7	0
Necropsy body wt	31.9 ± 1.2	33.1 ± 1.2	31.0 ± 0.8	30.6 ± 1.1	28.6 ± 0.8	
Heart						
Absolute	0.131 ± 0.004	0.156 ± 0.008*	0.135 ± 0.005	0.136 ± 0.006	0.126 ± 0.006	
Relative	4.127 ± 0.158	4.746 ± 0.261	4.381 ± 0.225	4.484 ± 0.236	4.388 ± 0.162	
R. Kidney						
Absolute	0.163 ± 0.004	0.166 ± 0.002	0.159 ± 0.003	0.160 ± 0.003	0.158 ± 0.002	
Relative	5.152 ± 0.154	5.061 ± 0.108	5.147 ± 0.134	5.274 ± 0.136	5.534 ± 0.170	
Liver						
Absolute	1.202 ± 0.045	1.309 ± 0.032	1.217 ± 0.029	1.180 ± 0.037	1.206 ± 0.043	
Relative	37.795 ± 1.035	39.733 ± 0.770	39.318 ± 0.991	38.710 ± 1.146	42.339 ± 2.101*	
Lung						
Absolute	0.297 ± 0.015	0.295 ± 0.014	0.295 ± 0.012	0.278 ± 0.013	0.239 ± 0.015**	
Relative	9.290 ± 0.279	9.016 ± 0.478	9.604 ± 0.557	9.108 ± 0.375	8.396 ± 0.578	
Spleen						
Absolute	0.080 ± 0.003	0.086 ± 0.002	0.081 ± 0.001	0.076 ± 0.002	0.074 ± 0.003	
Relative	2.525 ± 0.092	2.614 ± 0.074	2.616 ± 0.065	2.496 ± 0.089	2.593 ± 0.077	
Thymus						
Absolute	0.054 ± 0.004	0.057 ± 0.002	0.055 ± 0.003	0.050 ± 0.003	0.045 ± 0.004	
Relative	1.691 ± 0.111	1.746 ± 0.063	1.766 ± 0.110	1.626 ± 0.094	1.575 ± 0.142	

* 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] No data were available for the 60 mg/kg groups due to 100% mortality.
[c] n=9

APPENDIX F
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE F1 Summary of Reproductive Tissue Evaluations for Male Mice in the 14-Week Gavage Study of Acrylonitrile 176
TABLE F2 Estrous Cycle Characterization for Female Mice in the 14-Week Gavage Study of Acrylonitrile 176

Table F1
Summary of Reproductive Tissue Evaluations for Male Mice in the 14-Week Gavage Study of Acrylonitrile[a]

	Vehicle Control	5 mg/kg	10 mg/kg	20 mg/kg
n	9	10	10	10
Weights (g)				
Necropsy body wt	35.0 ± 1.7	34.2 ± 0.6	34.8 ± 0.8	32.2 ± 0.8
L. Cauda epididymis	0.0109 ± 0.0004	0.0117 ± 0.0003	0.0125 ± 0.0004**	0.0125 ± 0.0004**
L. Epididymis	0.0382 ± 0.0009	0.0403 ± 0.0005	0.0394 ± 0.0013	0.0404 ± 0.0008
L. Testis	0.1084 ± 0.0018	0.1100 ± 0.0019	0.1109 ± 0.0022	0.1095 ± 0.0022
Spermatid measurements				
Spermatid heads (10^7/g testis)	16.16 ± 0.56	16.20 ± 0.63	14.78 ± 0.72	15.94 ± 0.57
Spermatid heads (10^7/testis)	1.75 ± 0.07	1.78 ± 0.08	1.63 ± 0.07	1.75 ± 0.07
Spermatid count (mean/10^{-4} mL suspension)	54.72 ± 2.03	55.68 ± 2.36	51.00 ± 2.26	54.58 ± 2.32
Epididymal spermatozoal measurements				
Motility (%)	75.11 ± 0.56	75.60 ± 0.56	75.90 ± 0.66	75.80 ± 0.44
Concentration (10^6/g cauda epididymal tissue)	994 ± 145	946 ± 94	1,106 ± 107	980 ± 110

** Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' test
[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (necropsy body, left testis, and left epididymis weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

Table F2
Estrous Cycle Characterization for Female Mice in the 14-Week Gavage Study of Acrylonitrile[a]

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg
n	10	10	10	7
Necropsy body wt	31.9 ± 1.2	31.0 ± 0.8	30.6 ± 1.1	28.6 ± 0.8
Estrous cycle length (days)	4.20 ± 0.11	4.30 ± 0.11	4.33 ± 0.19[b]	4.57 ± 0.13
Estrous stages (% of cycle)				
Diestrus	22.5	22.5	20.8	27.4
Proestrus	25.0	22.5	20.8	21.4
Estrus	25.8	29.2	28.3	23.8
Metestrus	26.7	25.8	30.0	27.4

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (necropsy body weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females did 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 1 of 10 animals.

APPENDIX G
URINARY METABOLITE ANALYSES

Table G1 Urinary Metabolite Analyses Data for Mice at 2 Weeks and at 3, 12, and 18 Months in the 2-Year Gavage Study of Acrylonitrile 178

Table G1
Urinary Metabolite Analyses Data for Mice at 2 Weeks and at 3, 12, and 18 Months in the 2-Year Gavage Study of Acrylonitrile[a]

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Male				
n				
Week 2	5	5	5	5
Month 3	5	5	4	4
Month 12	5	5	5	5
Month 18	5	5	5	5
Volume (mL/24 hr)				
Week 2	0.8 ± 0.1	1.3 ± 0.2	1.1 ± 0.2	1.6 ± 0.1**
Month 3	1.2 ± 0.2	1.1 ± 0.2	1.0 ± 0.3	1.5 ± 0.3
Month 12	2.8 ± 0.4	2.2 ± 0.3	2.3 ± 0.2	2.0 ± 0.3
Month 18	2.6 ± 0.3	2.8 ± 0.7	3.0 ± 0.4	1.8 ± 0.4
Creatinine (mg/dL)				
Week 2	46.22 ± 1.27	44.74 ± 3.38	44.14 ± 4.54	40.90 ± 3.82
Month 3	36.98 ± 3.33	32.36 ± 4.22	30.30 ± 5.68	41.68 ± 6.25
Month 12	21.36 ± 2.07	27.00 ± 1.69	28.94 ± 3.00	27.76 ± 3.31
Month 18	25.78 ± 4.04	25.66 ± 2.90	26.08 ± 2.16	24.84 ± 2.76
Thiocyanate (µmol/L)				
Week 2	660.6 ± 36.7	1,005.4 ± 47.5**	1,582.2 ± 264.5**	2,876.0 ± 229.5**
Month 3	491.8 ± 69.3	890.2 ± 109.8*	2,131.8 ± 509.3**	3,991.8 ± 338.6**
Month 12	343.8 ± 37.1	835.0 ± 50.2**	2,297.8 ± 135.0**	4,362.8 ± 195.1**
Month 18	258.6 ± 12.5	751.0 ± 100.7**	2,118.4 ± 173.7**	4,168.2 ± 369.3**
Thiocyanate (µmol/mg creatinine)				
Week 2	1.4 ± 0.2	2.2 ± 0.2*	3.6 ± 0.7*	7.2 ± 0.7**
Month 3	1.2 ± 0.2	2.6 ± 0.2*	7.3 ± 0.9**	9.8 ± 1.0**
Month 12	1.6 ± 0.2	3.2 ± 0.2**	8.2 ± 0.9**	16.2 ± 1.4**
Month 18	1.0 ± 0.0	3.0 ± 0.0**[b]	8.0 ± 0.8**[b]	17.6 ± 2.2**
N-Acetyl-S-(2-cyanoethyl)-L-cysteine (µg/mL)				
Week 2	0.0 ± 0.0	27.4 ± 2.6**	114.5 ± 28.4**	241.4 ± 47.8**
Month 3	0.0 ± 0.0	56.3 ± 7.4**	296.1 ± 55.3**	436.2 ± 101.1**
Month 12	0.0 ± 0.0	57.6 ± 4.8**	248.8 ± 16.9**	509.8 ± 52.7**
Month 18	0.0 ± 0.0	34.5 ± 7.9**	175.4 ± 14.4**	488.7 ± 54.4**
N-Acetyl-S-(2-cyanoethyl)-L-cysteine (µmol/mg creatinine)				
Week 2	0.0 ± 0.0	0.3 ± 0.0**	1.2 ± 0.3**	2.5 ± 0.3**
Month 3	0.0 ± 0.0	0.8 ± 0.2**	5.2 ± 1.8**	4.5 ± 0.8**
Month 12	0.0 ± 0.0	0.9 ± 0.1**	3.9 ± 0.6**	8.1 ± 0.8**
Month 18	0.0 ± 0.0	0.6 ± 0.1**	2.9 ± 0.1**	8.7 ± 0.9**

Table G1
Urinary Metabolite Analyses Data for Mice at 2 Weeks and at 3, 12, and 18 Months in the 2-Year Gavage Study of Acrylonitrile

	Vehicle Control	2.5 mg/kg	10 mg/kg	20 mg/kg
Female				
n				
Week 2	5	4	4	5
Month 3	5	5	4	5
Month 12	5	5	5	5
Month 18	5	5	5	5
Volume (mL/24 hr)				
Week 2	1.5 ± 0.4	1.2 ± 0.1	1.3 ± 0.0	1.4 ± 0.2
Month 3	1.4 ± 0.1	1.0 ± 0.2	1.1 ± 0.2	1.4 ± 0.1
Month 12	1.9 ± 0.2	2.2 ± 0.3	2.2 ± 0.2	1.9 ± 0.2
Month 18	2.5 ± 0.5	2.7 ± 0.2	2.3 ± 0.6	2.7 ± 0.1
Creatinine (mg/dL)				
Week 2	38.12 ± 5.61	42.93 ± 2.97	42.38 ± 2.24	41.88 ± 2.89
Month 3	48.32 ± 2.18	62.82 ± 11.16	53.83 ± 6.15	42.66 ± 3.34
Month 12	22.10 ± 1.50	21.60 ± 1.93	23.98 ± 2.33	22.96 ± 4.03
Month 18	18.52 ± 1.05	19.62 ± 2.13	22.18 ± 2.88	16.40 ± 1.14
Thiocyanate (µmol/L)				
Week 2	481.6 ± 70.4	753.5 ± 35.3*	1,675.5 ± 68.8**	2,459.8 ± 258.8**
Month 3	368.6 ± 13.0	905.2 ± 101.6**	2,594.3 ± 155.4**	2,076.0 ± 164.9**
Month 12	274.6 ± 18.2	641.4 ± 41.5**	2,679.4 ± 202.6**	5,072.8 ± 289.6**
Month 18	170.0 ± 12.4	576.6 ± 52.2**	2,094.2 ± 126.0**	3,885.0 ± 218.1**
Thiocyanate (µmol/mg creatinine)				
Week 2	1.0 ± 0.0[b]	1.8 ± 0.3*	4.0 ± 0.4**	6.0 ± 0.5**
Month 3	1.0 ± 0.0	1.6 ± 0.2*	4.8 ± 0.3**	5.0 ± 0.8**
Month 12	1.0 ± 0.0	3.0 ± 0.0**	11.2 ± 0.4**	24.4 ± 3.2**
Month 18	1.0 ± 0.0[b]	2.8 ± 0.2**	10.8 ± 0.5**[b]	23.8 ± 1.3**
N-Acetyl-S-(2-cyanoethyl)-L-cysteine (µg/mL)				
Week 2	0.0 ± 0.0	25.8 ± 3.4**	87.1 ± 6.8**	184.7 ± 24.0**
Month 3	0.0 ± 0.0	36.4 ± 9.1**	125.5 ± 27.9**	154.7 ± 10.6**
Month 12	0.0 ± 0.0	40.6 ± 2.5**	193.2 ± 12.5**	390.4 ± 32.6**
Month 18	0.0 ± 0.0	33.9 ± 2.1**	174.9 ± 23.8**[b]	244.2 ± 36.7**
N-Acetyl-S-(2-cyanoethyl)-L-cysteine (µmol/mg creatinine)				
Week 2	0.0 ± 0.0	0.3 ± 0.0**	0.9 ± 0.1**	1.9 ± 0.2**
Month 3	0.0 ± 0.0	0.3 ± 0.1**	1.1 ± 0.3**	1.6 ± 0.1**
Month 12	0.0 ± 0.0	0.8 ± 0.1**	3.6 ± 0.5**	8.1 ± 1.3**
Month 18	0.0 ± 0.0	0.8 ± 0.1**	3.9 ± 0.4**[b]	6.4 ± 0.9**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=4

APPENDIX H
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

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CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

Procurement and Characterization of Acrylonitrile

Acrylonitrile was obtained from Aldrich Chemical Company (Milwaukee, WI) in two lots (02520DG and 00103TQ). Lot 02520DG was used during the 14-week and 2-year studies; lot 00103TQ was used during the 2-year study. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratories (Radian Corporation, Austin, TX and Battelle Memorial Institute, Columbus, OH) and the study laboratory. Reports on analyses performed in support of the acrylonitrile studies are on file at the National Institute of Environmental Health Sciences.

Lot 02520DG of the chemical, a colorless liquid, was identified as acrylonitrile by the analytical chemistry laboratory using infrared, ultraviolet/visible, and proton nuclear magnetic resonance spectroscopy and gas chromatography/mass spectroscopy (system A, Table H1) and by the study laboratory using infrared spectroscopy. Lot 00103TQ was identified as acrylonitrile by the analytical laboratory using infrared spectroscopy and proton and carbon-13 nuclear magnetic resonance spectroscopy. All spectra were consistent with the literature spectra (*Sadtler Standard Spectra; Sadtler Handbook of Infrared Spectra,* 1978; *Aldrich,* 1981, 1992; *NB575K Spectral Library*) and with the structure of acrylonitrile. The infrared and nuclear magnetic resonance spectra are presented in Figures H1 and H2.

The purity of lot 02520DG was determined by the analytical chemistry laboratory using Karl Fischer water analysis and gas chromatography with systems B and C and by the study laboratory using gas chromatography with system D with *n*-butanol added as an internal standard. The purity of lot 00103TQ was determined by the analytical laboratory using gas chromatography with systems E and F; elemental analyses and Karl Fischer water analysis were performed by Galbraith Laboratories, Inc. (Knoxville, TN). Major peak comparisons of lots 02520DG and 00103TQ were performed with gas chromatography by system E.

For lot 02520DG, Karl Fischer water analysis indicated 0.18% water. Gas chromatography by systems B and C indicated one major peak and no impurities. Gas chromatography by system D indicated a purity of 99.5% relative to a frozen reference standard. The overall purity was determined to be greater than 99%.

For lot 00103TQ, elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for acrylonitrile. Karl Fischer water analysis indicated approximately 0.6% water. Gas chromatography by systems E and F indicated one major peak and no impurities with areas of 0.1% or greater relative to the major peak area. Major peak comparisons indicated essentially identical purity profiles and retention times for lots 02520DG and 00103TQ. The overall purity of lot 00103TQ was determined to be greater than 99%.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using gas chromatography (system B). These studies indicated that acrylonitrile was stable as a bulk chemical for 14 days when stored protected from light and air at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature, protected from light, in amber glass bottles. Stability was monitored during the studies using gas chromatography (system D). No degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared every 4 weeks during the 14-week and 2-year studies by mixing acrylonitrile with deionized water (Table H2). The dose formulations were stored in amber glass bottles at room temperature (14-week study) or at approximately 5° C in amber glass bottles with Teflon®-lined septa that were sealed with metal crimp tops (2-year study) for up to 35 days.

Stability studies of a 0.8145 mg/mL formulation were performed by the analytical chemistry laboratory using gas chromatography with system B. Stability was confirmed for at least 35 days for dose formulations stored in sealed glass vials with Teflon®-lined caps at temperatures up to 28° C.

During the 14-week study, the dose formulations were analyzed at the beginning, midpoint, and end of the study; animal room samples of these dose formulations were also analyzed (Table H3). Of the dose formulations analyzed, all 15 were within 10% of the target concentrations, with no value greater than 108% of the target concentration; of the animal room samples analyzed, 7 of 15 were within 10% of the target concentrations. During the 2-year study, the dose formulations were analyzed approximately every 8 to 12 weeks; animal room samples of these dose formulations were also analyzed (Table H4). Of the dose formulations analyzed, 38 of 39 were within 10% of the target concentrations; of the animal room samples analyzed, all 12 were within 10% of the target concentrations. One dose formulation was 114% of the target concentration; it was remixed, and the new dose formulation was found to be within 10% of the target concentration.

Table H1
Gas Chromatography Systems Used in the Gavage Studies of Acrylonitrile[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Mass spectrometry	DB-624, 75 m × 0.53 mm (J&W Scientific, Folsom, CA)	Helium at 10 mL/minute	0° C for 10 minutes, then 5° C/minute to 225° C, held 4 minutes
System B			
Flame ionization	DB-17, 30 m × 0.53 mm (J&W Scientific)	Helium at 3.33 mL/minute	40° C for 5 minutes, then 10° C/minute to 90° C
System C			
Flame ionization	DB-WAX, 15 m × 0.53 mm (J&W Scientific)	Helium at 4.0 mL/minute	40° C for 3 minutes, then 10° C/minute to 90° C, held 2 minutes
System D			
Flame ionization	1% SP1000 on 60/80 Carbopack B, 2.4 m × 2 mm	Helium at 10 mL/minute	Isothermal at 150° C
System E			
Flame ionization	1% SP1000 on 60/80 Carbopack B, 2.4 m × 2 mm	Helium at 10 mL/minute	40° C to 210° C at 10° C/minute, held 11 minutes
System F			
Flame ionization	Vocol, 30 m × 0.25 mm (Supelco, Inc., Bellefonte, PA)	Helium at 3 mL/minute	35° C for 5 minutes, then 10° C/minute to 200° C, held 2 minutes

[a] Gas chromatographs were manufactured by Hewlett-Packard (Palo Alto, CA) (systems A, D, E, and F) and Varian, Inc. (Palo Alto, CA) (systems B and C).

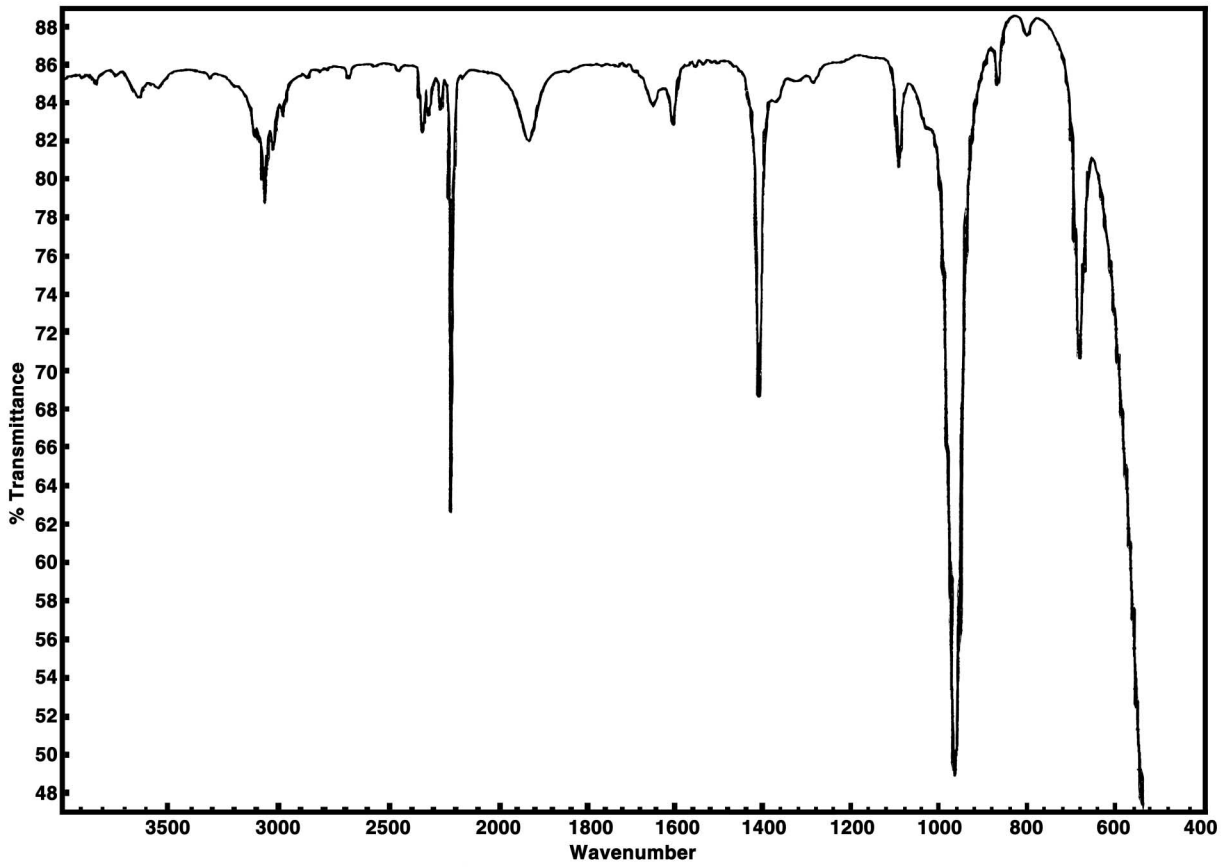

FIGURE H1
Infrared Absorption Spectrum of Acrylonitrile

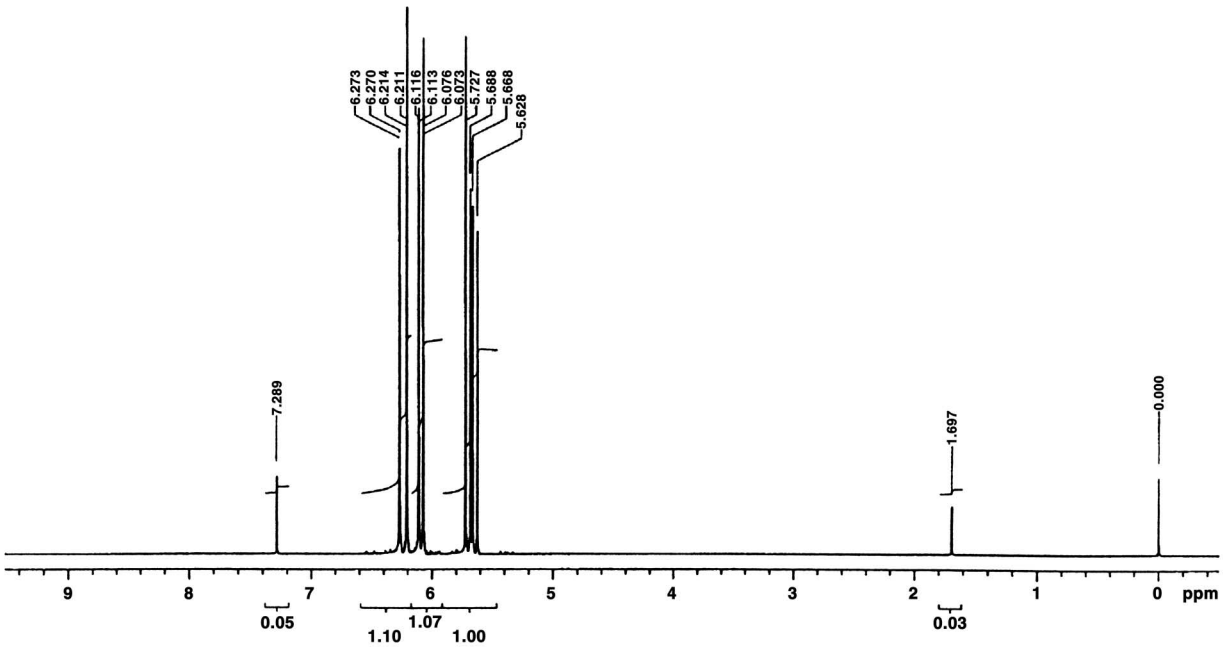

FIGURE H2
Nuclear Magnetic Resonance Spectrum of Acrylonitrile

Table H2
Preparation and Storage of Dose Formulations in the Gavage Studies of Acrylonitrile

14-Week Study	2-Year Study
Preparation	
Acrylonitrile was added to deionized water and stirred with a stir bar to form a solution. The doses were prepared every 4 weeks.	Acrylonitrile was added to deionized water and shaken or stirred to form a solution. The doses were prepared every 4 weeks.
Chemical Lot Number	
02520DG	02520DG and 00103TQ
Maximum Storage Time	
35 days	35 days
Storage Conditions	
Stored in amber glass bottles at room temperature	Stored at approximately 5° C in narrow-mouth, amber glass jars until dispensing; stored in amber glass bottles with Teflon®-lined septa sealed with metal crimp tops thereafter
Study Laboratory	
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)

Table H3
Results of Analyses of Dose Formulations Administered to Mice in the 14-Week Gavage Study of Acrylonitrile

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
14 November 1995	16 November 1995	0.5	0.4934	–1
		1	0.9894	–1
		2	1.955	–2
		4	3.902	–2
		6	5.755	–4
	18 November 1995[b]	0.5	0.3896	–22
		1	0.8892	–11
		2	1.555	–22
		4	3.030	–24
		6	5.580	–7
9 January 1996	10 January 1996	0.5	0.4844	–3
		1	1.008	+1
		2	1.982	–1
		4	3.980	0
		6	5.932	–1
	13 February 1996[b]	0.5	0.4823	–4
		1	0.9125	–9
		2	1.378	–31
		4	3.283	–18
		6	5.346	–11
6 February 1996	6 February 1996	0.5	0.5407	+8
		1	1.024	+2
		2	1.988	–1
		4	4.047	+1
		6	5.554	–7
	22 February 1996[b]	0.5	0.5594	+12
		1	1.039	+4
		2	2.054	+3
		4	4.343	+9
		6	6.366	+6

[a] Results of duplicate analyses. Dosing volume=10 mL/kg; 0.5 mg/mL=5 mg/kg, 1 mg/mL=10 mg/kg, 2 mg/mL=20 mg/kg, 4 mg/mL=40 mg/kg, 6 mg/mL=60 mg/kg
[b] Animal room samples

Table H4
Results of Analyses of Dose Formulations Administered to Mice in the 2-Year Gavage Study of Acrylonitrile

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
26 February 1997	26 February 1997	0.25	0.2564	+3
		1	1.046	+5
		2	2.084	+4
	2 April 1997[b]	0.25	0.2415	–3
		1	1.028	+3
		2	1.921	–4
22 April 1997	22 April 1997	0.25	0.2641	+6
		1	1.054	+5
		2	2.108	+5
18 June 1997	23 June 1997	0.25	0.2565	+3
		1	1.046	+5
		2	2.092	+5
10 September 1997	15 September 1997	0.25	0.2552	+2
		1	1.033	+3
		2	2.029	+1
	16 October 1997[b]	0.25	0.2557	+2
		1	1.017	+2
		2	2.037	+2
5 November 1997	7 November 1997	0.25	0.2499	0
		1	1.023	+2
		2	2.154	+8
31 December 1997	31 December 1997	0.25	0.2596	+4
		1	1.022	+2
		2	2.193	+10
25 March 1998	2 April 1998	0.25	0.2471	–1
		1	0.9909	–1
		2	2.034	+2
	30 April 1998[b]	0.25	0.2491	0
		1	0.9439	–6
		2	2.035	+2
20 May 1998	21 May 1998	0.25	0.2703	+8
		1	1.051	+5
		2	2.144	+7
15 July 1998	16 July 1998	0.25	0.2535	+1
		1	1.015	+2
		2	2.154	+8

Table H4
Results of Analyses of Dose Formulations Administered to Mice in the 2-Year Gavage Study of Acrylonitrile

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
7 October 1998	8 October 1998	0.25	0.2562	+2
		1	0.9820	–2
		2	1.999	0
	11 November 1998[b]	0.25	0.2278	–9
		1	0.9219	–8
		2	2.034	+2
2 December 1998	2 December 1998	0.25	0.2648	+6
		1	1.046	+5
		2	2.286[c]	+14
4 December 1998	4 December 1998	2	1.943[d]	–3
30 December 1998	31 December 1998	0.25	0.2483	–1
		1	0.9780	–2
		2	1.960	–2
27 January 1999	28 January 1999	0.25	0.2458	–2
		1	0.9426	–6
		2	1.936	–3

[a] Results of duplicate analyses. Dosing volume=10 mL/kg; 0.25 mg/mL=2.5 mg/kg, 1 mg/mL=10 mg/kg, 2 mg/mL=20 mg/kg
[b] Animal room samples
[c] Remixed; not used in study
[d] Results of remix

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

TABLE I1 Ingredients of NTP-2000 Rat and Mouse Ration 192
TABLE I2 Vitamins and Minerals in NTP-2000 Rat and Mouse Ration 192
TABLE I3 Nutrient Composition of NTP-2000 Rat and Mouse Ration 193
TABLE I4 Contaminant Levels in NTP-2000 Rat and Mouse Ration 194

TABLE I1
Ingredients of NTP-2000 Rat and Mouse Ration

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

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

TABLE I2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

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

[a] Per kg of finished product

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

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	13.1 ± 0.36	12.5 – 13.8	24
Crude fat (% by weight)	8.1 ± 0.25	7.6 – 8.4	24
Crude fiber (% by weight)	9.5 ± 0.59	8.0 – 10.3	24
Ash (% by weight)	5.0 ± 0.15	4.7 – 5.3	24
Amino Acids (% of total diet)			
Arginine	0.731 ± 0.050	0.670 – 0.800	8
Cystine	0.224 ± 0.012	0.210 – 0.240	8
Glycine	0.684 ± 0.041	0.620 – 0.740	8
Histidine	0.333 ± 0.018	0.310 – 0.350	8
Isoleucine	0.524 ± 0.046	0.430 – 0.590	8
Leucine	1.061 ± 0.061	0.960 – 1.130	8
Lysine	0.708 ± 0.056	0.620 – 0.790	8
Methionine	0.401 ± 0.035	0.350 – 0.460	8
Phenylalanine	0.598 ± 0.036	0.540 – 0.640	8
Threonine	0.501 ± 0.051	0.430 – 0.590	8
Tryptophan	0.126 ± 0.014	0.110 – 0.150	8
Tyrosine	0.390 ± 0.056	0.280 – 0.460	8
Valine	0.640 ± 0.049	0.550 – 0.690	8
Essential Fatty Acids (% of total diet)			
Linoleic	3.97 ± 0.284	3.59 – 4.54	8
Linolenic	0.30 ± 0.042	0.21 – 0.35	8
Vitamins			
Vitamin A (IU/kg)	5,189 ± 1,145	3,280 – 7,420	24
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	82.2 ± 14.08	62.2 – 107.0	8
Thiamine (ppm)	7.5 ± 0.94	6.0 – 9.3	24
Riboflavin (ppm)	5.6 ± 1.12	4.20 – 7.70	8
Niacin (ppm)	74.3 ± 5.94	66.4 – 85.8	8
Pantothenic acid (ppm)	22.5 ± 3.96	17.4 – 29.1	8
Pyridoxine (ppm)	9.04 ± 2.37	6.4 – 12.4	8
Folic acid (ppm)	1.64 ± 0.38	1.26 – 2.32	8
Biotin (ppm)	0.333 ± 0.15	0.225 – 0.704	8
Vitamin B_{12} (ppb)	68.7 ± 63.0	18.3 – 174.0	8
Choline (ppm)	3,155 ± 325	2,700 – 3,790	8
Minerals			
Calcium (%)	0.969 ± 0.040	0.905 – 1.050	24
Phosphorus (%)	0.544 ± 0.024	0.496 – 0.580	24
Potassium (%)	0.659 ± 0.022	0.627 – 0.691	8
Chloride (%)	0.357 ± 0.027	0.300 – 0.392	8
Sodium (%)	0.189 ± 0.019	0.160 – 0.212	8
Magnesium (%)	0.199 ± 0.009	0.185 – 0.213	8
Sulfur (%)	0.178 ± 0.021	0.153 – 0.209	8
Iron (ppm)	160 ± 14.7	135 – 177	8
Manganese (ppm)	50.3 ± 4.82	42.1 – 56.0	8
Zinc (ppm)	50.7 ± 6.59	43.3 – 61.1	8
Copper (ppm)	6.29 ± 0.828	5.08 – 7.59	8
Iodine (ppm)	0.461 ± 0.187	0.233 – 0.843	8
Chromium (ppm)	0.724 ± 0.529	0.330 – 2.000	8
Cobalt (ppm)	0.45 ± 0.628	0.20 – 2.0	8

[a] From formulation

Table I4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.22 ± 0.143	0.10 – 0.50	24
Cadmium (ppm)	0.04 ± 0.012	0.04 – 0.10	24
Lead (ppm)	0.08 ± 0.020	0.06 – 0.15	24
Mercury (ppm)	<0.02		24
Selenium (ppm)	0.16 ± 0.028	0.11 – 0.23	24
Aflatoxins (ppb)	<5.00		24
Nitrate nitrogen (ppm)[c]	16.4 ± 8.11	9.04 – 39.6	24
Nitrite nitrogen (ppm)[c]	<0.61		24
BHA (ppm)[d]	1.1 ± 0.37	1.00 – 2.47	24
BHT (ppm)[d]	1.0 ± 0.14	1.00 – 1.68	24
Aerobic plate count (CFU/g)[e]	10 ± 1	10 – 15	24
Coliform (MPN/g)[e]	0.0	0 – 3	24
Escherichia coli (MPN/g)	<10		24
Salmonella (MPN/g)	Negative		24
Total nitrosoamines (ppb)[f]	5.5 ± 3.77	2.1 – 20.9	24
N-Nitrosodimethylamine (ppb)[f]	2.1 ± 1.31	1.0 – 6.4	24
N-Nitrosopyrrolidine (ppb)[f]	3.5 ± 2.73	1.0 – 14.5	24
Pesticides (ppm)			
α-BHC	<0.01		24
β-BHC	<0.02		24
γ-BHC	<0.01		24
δ-BHC	<0.01		24
Heptachlor	<0.01		24
Aldrin	<0.01		24
Heptachlor epoxide	<0.01		24
DDE	<0.01		24
DDD	<0.01		24
DDT	<0.01		24
HCB	<0.01		24
Mirex	<0.01		24
Methoxychlor	<0.05		24
Dieldrin	<0.01		24
Endrin	<0.01		24
Telodrin	<0.01		24
Chlordane	<0.05		24
Toxaphene	<0.10		24
Estimated PCBs	<0.20		24
Ronnel	<0.01		24
Ethion	<0.02		24
Trithion	<0.05		24
Diazinon	<0.10		24
Methyl chlorpyrifos	0.107 ± 0.088	0.020 – 0.368	24
Methyl parathion	<0.02		24
Ethyl parathion	<0.02		24
Malathion	0.322 ± 0.571	0.020 – 2.810	24
Endosulfan I	<0.01		24
Endosulfan II	<0.01		24
Endosulfan sulfate	<0.03		24

[a] CFU=colony-forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride
[b] For values less than the limit of detection, the detection limit is given as the mean.
[c] Sources of contamination: alfalfa, grains, and fish meal
[d] Sources of contamination: soy oil and fish meal
[e] All samples were irradiated; microbial counts for 23 of 24 samples were below the detection limit.
[f] All values were corrected for percent recovery.

APPENDIX J
SENTINEL ANIMAL PROGRAM

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Results 197

SENTINEL ANIMAL PROGRAM

METHODS

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

Serum samples were collected from randomly selected mice during the 14-week and 2-year studies. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to BioReliance Corporation (Microbiological Associates, Inc.) (Rockville, MD) for determination of antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Method and Test	Time of Analysis
14-Week Study	
ELISA	
Ectromelia virus	4 weeks, study termination
EDIM (epizootic diarrhea of infant mice)	4 weeks, study termination
GDVII (mouse encephalomyelitis virus)	4 weeks, study termination
LCM (lymphocytic choriomeningitis virus)	4 weeks, study termination
Mouse adenoma virus-FL	4 weeks, study termination
MHV (mouse hepatitis virus)	4 weeks, study termination
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	Study termination
PVM (pneumonia virus of mice)	4 weeks, study termination
Reovirus 3	4 weeks, study termination
Sendai	4 weeks, study termination
Immunofluorescence Assay	
MCMV (mouse cytomegalovirus)	Study termination
Hemagglutination Inhibition	
K (papovavirus)	4 weeks, study termination
MVM (minute virus of mice)	4 weeks, study termination
Polyoma virus	4 weeks, study termination

2-Year Study

ELISA	
Ectromelia virus	4 weeks, 6, 12, and 18 months, study termination
EDIM	4 weeks, 6, 12, and 18 months, study termination
GDVII	4 weeks, 6, 12, and 18 months, study termination
LCM	4 weeks, 6, 12, and 18 months, study termination
Mouse adenoma virus-FL	4 weeks, 6, 12, and 18 months, study termination
MHV	4 weeks, 6, 12, and 18 months, study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	4 weeks, 6, 12, and 18 months, study termination
Reovirus 3	4 weeks, 6, 12, and 18 months, study termination
Sendai	4 weeks, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
Mouse adenoma virus-FL	12 and 18 months
MCMV	Study termination
MHV	Study termination
Parvovirus	12 and 18 months, study termination
PVM	12 and 18 months, study termination
Hemagglutination Inhibition	
K	4 weeks, 6 months
MVM	4 weeks, 6 months
Polyoma virus	4 weeks, 6 months

Results

All test results were negative.