NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF ACRYLAMIDE
(CAS No. 79-06-1)

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

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

July 2012

NTP TR 575

NIH Publication No. 12-5917

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

FOREWORD

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CONTRIBUTORS

This study on acrylamide was conducted at the Food and Drug Administration's (FDA) National Center for Toxicological Research (NCTR) under an interagency agreement between the FDA and the National Institute of Environmental Health Sciences (NIEHS). The studies were designed and monitored by a Toxicology Study Selection and Review Committee composed of representatives from the NCTR and other FDA centers, NIEHS, and other *ad hoc* members from other government agencies and academia. The interagency agreement was designed to use the staff and facilities of the NCTR in the testing of FDA priority chemicals and to provide FDA scientists and regulatory policymakers information for hazard identification and risk assessment.

National Center for Toxicological Research, Food and Drug Administration

Conducted study, evaluated and interpreted results and pathology findings, reported findings, and prepared the study report

F.A. Beland, Ph.D., Study Scientist
D.R. Doerge, Ph.D., Co-Investigator
L.P. McDaniel, B.S., Study Coordinator

Conducted microbiology surveillance and diagnostics

R.M. Colvert, B.S.
M.A. Holland, B.S.
D.D. Paine, B.S.
L.M. Sims, B.S.
R.S. Steele, B.S.
C.V. Summage-West, B.S.
R.D. Wagner, Ph.D.

Conducted dose certifications and chemical analyses

P.H. Siitonen, B.S.
S.M. Billedeau, M.S.
C.R. Cozart, B.S.
F.E. Evans, Ph.D.
J.P. Freeman, Ph.D.
T.M. Heinze, M.S.
J.J. James, B.S.

Conducted statistical analyses

M.B. Mendoza, Ph.D.

Conducted quality assurance audits

J.M. Fowler, B.S.
Y.E. Whiteside, B.S.

Prepared technical report

R.L. Stingley, Ph.D., Project Leader
S.C. Matson, Ph.D.
A.R. Babb, B.S.

National Institute of Environmental Health Sciences

Reviewed and evaluated the technical report, interpreted results and pathology findings

N.J. Walker, Ph.D.
D.E. Malarkey, D.V.M., Ph.D.
P.M. Foster, Ph.D.
C.J. Alden, Ph.D.
G.S. Travlos, D.V.M.
G.E. Kissling, Ph.D.
J.K. Dunnick, Ph.D.
B.J. Collins, M.S.P.H.

Z-Tech Corporation

Provided software systems development and data entry

K.A. Carroll
B. Spadoni

Bionetics Corporation

Prepared dosed animal feed and water, and provided animal care

C. Cain
J. Carson, B.S.
A. Matson, B.S.
M. Nichols
M. Vanlandingham

Toxicologic Pathology Associates
Evaluated pathology findings

P.W. Mellick, D.V.M., Ph.D., Study Pathologist (Rat)
G.R. Olson, D.V.M., Ph.D., Study Pathologist (Mouse)
A. Warbritton
L.P. Wiley, B.S.

Experimental Pathology Laboratories, Inc.
Provided pathology review (May 2009)

R.A. Miller, D.V.M., Ph.D.,
Pathology Quality Assessment Pathologist
(Female Rats)
R.R. Maronpot, D.V.M., M.S., M.P.H.,
Pathology Quality Assessment Pathologist
(Male Rats)
J.F. Hardisty, D.V.M.
Pathology Quality Assessment Pathologist (Mice)

Provided neuropathology review (July 2009)

R.A. Miller, D.V.M., Ph.D.,
Pathology Quality Assessment Pathologist (Mouse)
G.A. Willson, B.V.M.S.
Pathology Quality Assessment Pathologist (Rat)

NTP Pathology Working Group
Evaluated slides and prepared pathology reports (June 2009)

R.A. Miller, D.V.M., Ph.D., Coordinator (Mouse)
Experimental Pathology Laboratories, Inc.
R.R. Maronpot, D.V.M., Coordinator (Rat)
Experimental Pathology Laboratories, Inc.
D.E. Malarkey, D.V.M., Ph.D.
National Institute of Environmental Health Sciences
G.R. Olson, D.V.M., Ph.D.
Toxicologic Pathology Associates
(Study Pathologist, Mouse)
P.W. Mellick, D.V.M., Ph.D.
Toxicologic Pathology Associates
(Study Pathologist, Rat)
J.R. Latendresse, D.V.M., Ph.D.
Toxicologic Pathology Associates
J.B. Nold, D.V.M., Ph.D., Consultant
WIL Biotechnics

Neuropathology Working Group
Evaluated slides and prepared pathology reports (September and October 2009)

M.P. Jokinen, D.V.M., Coordinator (Rat)
Charles Rivers Laboratories, Pathology Associates
J.P. Morrison, D.V.M., Ph.D., Coordinator (Mouse)
Charles Rivers Laboratories, Pathology Associates
P.B. Little, D.V.M.
Charles Rivers Laboratories, Pathology Associates
D.E. Malarkey, D.V.M., Ph.D.
National Institute of Environmental Health Sciences
R.C. Sills, D.V.M., Ph.D.
National Institute of Environmental Health Sciences
J.F. Hardisty, D.V.M.
Experimental Pathology Laboratories, Inc.
R.A. Miller, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
J.C. Peckham, D.V.M., M.S., Ph.D.
Experimental Pathology Laboratories, Inc.
G.A. Willson, B.V.M.S.
Experimental Pathology Laboratories, Inc.
R.R. Maronpot, D.V.M.
Experimental Pathology Laboratories, Inc.
J.B. Nold, D.V.M., Ph.D., Consultant
WIL Biotechnics
G.R. Olson, D.V.M., Ph.D.
Toxicologic Pathology Associates
(Study Pathologist, Mouse)
P.W. Mellick, D.V.M., Ph.D.
Toxicologic Pathology Associates
(Study Pathologist, Rat)
J.R. Latendresse, D.V.M., Ph.D.
Toxicologic Pathology Associates

NIEHS/FDA Interagency Agreement Project Officers

P.C. Howard, Ph.D.
National Center for Toxicological Research
W.T. Allaben, Ph.D.
National Center for Toxicological Research
N.J. Walker, Ph.D.
National Institute of Environmental Health Sciences
J.R. Bucher, Ph.D.
National Institute of Environmental Health Sciences

CONTENTS

SUMMARY

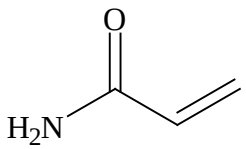
Background
Acrylamide is used in industry to produce polyacrylamides but it also is produced in the baking and frying of starchy foods, including french fries, potato chips, and bread. Acrylamide is already known to cause cancer in laboratory animals, and we studied the effects of acrylamide in male and female rats and mice to help identify the levels that produce potential toxic or cancer-related hazards.

Methods
We gave drinking water containing 6.25, 12.5, 25, or 50 parts per million (ppm) of acrylamide to groups of 50 male and female rats and mice for 2 years. Control animals received the same tap water with no chemical added. At the end of the study tissues from more than 40 sites were examined for every animal.

Results
Animals receiving 50 ppm acrylamide had lower survival rates than the controls. The rates of several types of cancer increased in each of the animal studies. Male and female rats receiving acrylamide had increased rates of thyroid gland and heart tumors; male rats also had increased rates of cancer in the pancreatic islets and of malignant mesotheliomas, and female rats also had increased rates of cancers in the clitoral gland, liver, mammary gland, skin, and mouth or tongue. Male and female mice had increased rates of cancer in the harderian gland, lung, and stomach; female mice also had increased rates of cancer in the mammary gland, skin, and ovary.

Conclusions
We conclude that acrylamide in the drinking water caused cancer in several different tissues in male and female rats and mice.

ABSTRACT



ACRYLAMIDE

CAS No. 79-06-1

Chemical Formula: C_3H_5NO Molecular Weight: 71.08

Synonyms: 2-Propenamide, acrylagel, acrylic acid amide, acrylic amide, ethylenecarboxamide, propenamide, vinyl amide.

Acrylamide, a water-soluble α,β-unsaturated amide, is a contaminant in baked and fried starchy foods, including french fries, potato chips, and bread, as a result of Maillard reactions involving asparagine and reducing sugars. Additional sources of acrylamide exposure include cigarettes, laboratory procedures involving polyacrylamide gels, and various occupations (*e.g*, monomer production and polymerization processes). Acrylamide is carcinogenic in experimental animals. To obtain data for developing quantitative risk assessments for dietary exposures to acrylamide, the Food and Drug Administration nominated acrylamide for an in-depth toxicological evaluation by the National Toxicology Program. As part of this evaluation, male and female B6C3F1/Nctr (C57BL/6N x C3H/HeN MTV$^-$) mice and male and female F344/N Nctr rats were exposed to acrylamide (at least 99.4% pure) in drinking water for 2 years.

2-WEEK STUDY IN RATS

Groups of four male and four female F344/N rats were administered 0, 0.14, 0.35, 0.70, 1.41, 3.52, or 7.03 mM acrylamide in the drinking water (0, 10, 25, 50, 100, 250, or 500 ppm acrylamide) or 0.0, 7.4, 18.5, 37, 74, 185, or 370 mg acrylamide per kg diet for 14 days. One male rat administered 7.03 mM acrylamide in the drinking water died on day 14. Male and female rats receiving 7.03 mM acrylamide weighed 56% and 64% of controls, respectively. Male and female rats fed 370 mg acrylamide per kg diet weighed 74% and 83% of controls, respectively. Female rats receiving 3.52 mM acrylamide in drinking water and male rats fed 185 mg acrylamide per kg diet weighed 85% and 89% of controls, respectively. Rats receiving 7.03 mM acrylamide in drinking water or 370 mg acrylamide per kg diet exhibited hind-leg paralysis on day 14. Mild to moderate dilatation of the urinary bladder was observed in all rats given 370 mg acrylamide per kg diet, and in three of four male rats and all four female rats given 7.03 mM acrylamide in drinking water, and in one of four male rats given 3.52 mM acrylamide in drinking water. Mild to moderate degeneration of the germinal epithelium in the seminiferous tubules of the testes was noted microscopically in all male rats given 7.03 mM acrylamide in drinking water and in two of four male rats fed 370 mg acrylamide per kg diet.

2-WEEK STUDY IN MICE

Groups of four male and four female B6C3F1 mice were administered 0, 0.14, 0.35, 0.70, 1.41, 3.52, or 7.03 mM acrylamide in the drinking water (0, 10, 25, 50, 100, 250, or 500 ppm acrylamide) or 0.0, 7.4, 18.5, 37, 74, 185, or 370 mg acrylamide per kg diet for 14 days. None of the mice administered 7.03 mM acrylamide in the drinking water survived the 14-day study. Mice administered 7.03 mM acrylamide in the drinking water showed marked decreases in body weight (greater than 25% compared to control mice) after seven days of treatment, and two of the mice displayed hind leg paralysis. No significant adverse

effects were observed in mice administered 3.52 mM acrylamide in the drinking water for 14 days.

Female B6C3F1 mice given 370 mg acrylamide per kg diet for 14 days showed a modest decrease (11%) in body weight. No other significant adverse effects were observed in mice administered any dose of acrylamide in the diet.

3-MONTH STUDY IN RATS

Groups of eight male and eight female F344/N rats were administered 0.0, 0.14, 0.35, 0.70, 1.41, or 3.52 mM acrylamide in the drinking water (0, 10, 25, 50, 100, or 250 ppm acrylamide) or 0.0, 7.4, 18.5, 37, 74, or 185 mg acrylamide per kg diet for 13 weeks. After 13 weeks, male and female rats administered 3.52 mM acrylamide weighed 73% and 71% of the control rats, respectively. Male and female rats fed 185 mg acrylamide per kg diet for 13 weeks weighed 86% and 82% of the control rats, respectively. Hind-leg paralysis was observed in all rats administered 3.52 mM acrylamide in the drinking water or 185 mg acrylamide per kg diet. Four of eight female rats administered 1.41 mM acrylamide also displayed hind-leg paralysis. Radiculoneuropathy (a degenerative lesion) involving the sciatic nerve and lumbar spinal cord was observed in all male and female rats administered 3.52 mM acrylamide or 185 mg acrylamide per kg diet. A low incidence of radiculoneuropathy was also noted in female rats fed 74 mg acrylamide per kg diet. The neuronal degenerative changes were accompanied, at times, by atrophy in skeletal muscle of the hind-limb and luminal dilation of the urinary bladder. All rats treated with 3.52 mM acrylamide displayed increased hemosiderin pigment in their spleens and hyperplasia of red blood cell precursors in their bone marrow. Two of eight male rats fed 185 mg acrylamide per kg diet also had increased hemosiderin pigment in their spleens.

Degeneration of the germ cells in the testes was observed in all male rats given 1.41 or 3.52 mM acrylamide, or 185 mg acrylamide per kg diet. A lower incidence of this lesion was also detected in all other doses of acrylamide in the diet.

3-MONTH STUDY IN MICE

Groups of eight male and eight female B6C3F1 mice were administered 0, 0.14, 0.35, 0.70, 1.41, or 3.52 mM acrylamide in the drinking water (0, 10, 25, 50, 100, or 250 ppm acrylamide) or 0.0, 18.5, 37, 74, 185, or 370 mg acrylamide per kg diet for 13 weeks. Two mice died before the end of the study: one male fed 185 mg acrylamide and one male fed 370 mg acrylamide per kg diet. After 13 weeks, the male and female mice given 3.52 mM acrylamide weighed 86% and 94% of their respective control mice; male mice administered 1.41 mM acrylamide weighed 91% of the control male mice; and male and female mice fed 370 mg acrylamide per kg diet weighed 87% and 81% of their respective control groups. Hind-limb paralysis was observed in all mice administered 3.52 mM acrylamide or 370 mg acrylamide per kg diet. Radiculoneuropathy involving the sciatic nerve, lumbar spinal cord, or both was observed in all male and female mice administered 3.52 mM acrylamide. Radiculoneuropathy, involving primarily the sciatic nerve, was also noted in one of eight female mice fed 185 mg acrylamide per kg diet and in mice fed 370 mg acrylamide per kg diet. The neuronal degenerative changes were accompanied, at times, by atrophy in skeletal muscle of the hind-limb and luminal dilation of the urinary bladder. Degeneration of the germ cells in the testes was observed in six of eight male mice given 3.52 mM acrylamide and seven of seven mice fed 370 mg acrylamide per kg diet.

2-YEAR STUDY IN RATS

Groups of 48 male and 48 female F344/N rats were administered acrylamide in the drinking water *ad libitum* for 2 years. Concentrations of 0.0875, 0.175, 0.35, and 0.70 mM acrylamide (6.25, 12.5, 25, and 50 ppm acrylamide) resulted in an average daily consumption of approximately 0.33, 0.66, 1.32, and 2.71 mg acrylamide per kg body weight in male F344/N rats and 0.44, 0.88, 1.84, and 4.02 mg acrylamide per kg body weight in female F344/N rats.

Acrylamide had no effect upon the survival of male F344/N rats. Female F344/N rats administered 0.175, 0.35, or 0.70 mM acrylamide had decreased survival compared to control female F344/N rats. Acrylamide caused significant dose-related decreasing trends in body weight in F344/N rats. At the end of the 2 year period, male and female F344/N rats administered 0.70 mM acrylamide weighed 86% and 85% of their respective control groups. Feed consumption was generally not affected by acrylamide; water consumption in female F344/N rats was increased at later time points.

In male F344/N rats, the incidence of epididymis malignant mesothelioma, combined epididymis or testicular tunica malignant mesothelioma, heart malignant incidences of schwannoma, pancreatic islets adenoma, thyroid gland follicular cell carcinoma, and combined thyroid gland follicular cell adenoma or carcinoma was increased significantly in the 0.70 mM acrylamide group.

In female F344/N rats, the incidence of clitoral gland carcinoma was increased significantly in the 0.0875, 0.175, and 0.70 mM acrylamide groups. The incidence of mammary gland fibroadenoma was increased significantly at 0.175, 0.35, and 0.70 mM acrylamide. Significant increases in neoplasm incidences were also observed in oral mucosa squamous cell papilloma, combined oral mucosa or tongue squamous cell papilloma or carcinoma, combined skin fibroma, fibrosarcoma, or sarcoma, and combined thyroid gland follicular cell adenoma or carcinoma at 0.70 mM acrylamide.

2-YEAR STUDY IN MICE

Groups of 48 male and 48 female B6C3F1 mice were administered acrylamide in the drinking water *ad libitum* for 2 years. Concentrations of 0.0875, 0.175, 0.35, and 0.70 mM acrylamide (6.25, 12.5, 25, and 50 ppm acrylamide) resulted in average daily consumption of approximately 1.04, 2.20, 4.11, and 8.93 mg acrylamide per kg body weight in male B6C3F1 mice and 1.10, 2.23, 4.65, and 9.96 mg acrylamide per kg body weight in female B6C3F1 mice.

Acrylamide caused dose-related decreasing trends in survival in B6C3F1 mice, with the survival being significantly decreased in male B6C3F1 mice administered 0.70 mM acrylamide and female B6C3F1 mice given 0.35 and 0.70 mM acrylamide. Acrylamide caused only sporadic changes in body weight in B6C3F1 mice, with the magnitude of the change never exceeding 6% of the respective control body weight. Food and water consumption was generally not affected by acrylamide, except for an increased consumption by female B6C3F1 mice in the 0.70 mM acrylamide group toward the end of the study.

In male B6C3F1 mice, the incidence of harderian gland adenoma and combined harderian gland adenoma or adenocarcinoma was increased significantly in all acrylamide dose groups. The incidence of lung alveolar/bronchiolar adenoma and combined lung alveolar/bronchiolar adenoma or carcinoma was increased significantly at 0.175 and 0.70 mM acrylamide, and the incidence of stomach (forestomach) squamous cell papilloma and combined stomach (forestomach) squamous cell papilloma or carcinoma was increased significantly at 0.35 and 0.70 mM acrylamide.

In female B6C3F1 mice, the incidence of harderian gland adenoma was increased significantly in all dosed groups. The combined incidence of mammary gland adenoacanthoma or adenocarcinoma was increased significantly at 0.175, 0.35, and 0.70 mM acrylamide, and the incidence of mammary gland adenocarcinoma was increased significantly at 0.175 and 0.70 mM acrylamide. Incidences of lung alveolar/bronchiolar adenoma, combined lung alveolar/bronchiolar adenoma or carcinoma, and malignant mesenchymal skin tumors (fibrosarcoma, hemangiosarcoma, liposarcoma, myxosarcoma, neurofibrosarcoma, or sarcoma) were increased significantly at 0.35 and 0.70 mM acrylamide. A significant increase was also observed in the incidence of ovary granulosa cell tumor (benign) and mammary gland adenoacanthoma at 0.70 mM.

CONCLUSIONS

Under the conditions of these 2-year drinking water studies, there was *clear evidence of carcinogenic activity* of acrylamide in male F344/N rats based on increased incidences of malignant mesothelioma of the epididymis and testis tunica, malignant schwannoma of the heart, and follicular cell adenoma or carcinoma of the thyroid gland. An increased incidence of pancreatic islet adenoma was also considered related to acrylamide exposure.

There was *clear evidence of carcinogenic activity* of acrylamide in female F344/N rats based on increased incidences of fibroadenoma of the mammary gland, squamous cell neoplasms (primarily papilloma) of the oral cavity (mucosa or tongue), mesenchymal neoplasms (fibroma, fibrosarcoma, or sarcoma) of the skin, and follicular cell neoplasms (adenoma or carcinoma) of the thyroid gland. Increased incidences of hepatocellular adenoma of the liver and carcinoma of the clitoral gland were also considered to be related to acrylamide exposure. The occurrence of malignant schwannoma of the heart may have been related to acrylamide exposure.

There was *clear evidence of carcinogenic activity* of acrylamide in male B6C3F1 mice based on increased incidences of neoplasms (primarily adenoma) of the harderian gland, alveolar/bronchiolar neoplasms (primarily adenoma) of the lung and squamous cell neoplasms (primarily papilloma) of the forestomach.

There was *clear evidence of carcinogenic activity* of acrylamide in female B6C3F1 mice based on increased incidences of harderian gland adenoma, alveolar/ bronchiolar adenoma of the lung, adenoacanthoma and adenocarcinoma of the mammary gland, benign granulosa cell neoplasms of the ovary, and malignant mesenchymal neoplasms of the skin. Increased incidences of squamous cell papilloma of the forestomach were also considered to be related to acrylamide exposure.

Exposure to acrylamide was associated with increased incidences of degeneration of the retina and sciatic nerve in male and female rats; preputial gland duct ectasia in male rats; adrenal cortex hypertrophy and cytoplasmic vacuolization, bone marrow hyperplasia, ovarian atrophy, and spleen hematopoietic cell proliferation in female rats; cataracts of the eye, spleen hematopoietic cell proliferation, and forestomach epithelial hyperplasia in male and female mice; preputial gland inflammation and lung epithelial hyperplasia in male mice; and ovarian cysts in female mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 13. A summary of the Peer Review Panel comments and the public discussion on this Technical Report appears on page 15.

Summary of the 2-Year Carcinogenesis Studies of Acrylamide

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Doses in drinking water	0, 0.0875, 0.175, 0.35, or 0.70 mM acrylamide (0, 6.25, 12.5, 25, or 50 ppm acrylamide) *ad libitum* for 2 years	0, 0.0875, 0.175, 0.35, or 0.70 mM acrylamide (0, 6.25, 12.5, 25, or 50 ppm acrylamide) *ad libitum* for 2 years	0, 0.0875, 0.175, 0.35, or 0.70 mM acrylamide (0, 6.25, 12.5, 25, or 50 ppm acrylamide) *ad libitum* for 2 years	0, 0.0875, 0.175, 0.35, or 0.70 mM acrylamide (0, 6.25, 12.5, 25, or 50 ppm acrylamide) *ad libitum* for 2 years
Body weights	0.70 mM acrylamide exposure group weighed 86% of control group after 2 years	0.70 mM acrylamide exposure group weighed 85% of control group after 2 years	Only sporadic changes, with magnitude ≤ 4% of controls.	Only sporadic changes, with magnitude ≤ 6% of controls.
Survival rates	17/48, 14/48, 19/48, 16/48, 9/48	34/48, 28/48, 21/48, 23/48, 13/48	39/48, 39/48, 37/48, 38/48, 28/48	39/48, 36/48, 36/48, 25/48, 15/48
Nonneoplastic effects	Eye: retina degeneration (2/44, 2/47, 3/47, 2/46, 10/45) Peripheral nerve (sciatic): axon degeneration (5/48, 7/48, 7/48, 11/48, 23/48) Preputial gland: duct ectasia (4/48, 6/47, 11/48, 14/48, 10/48)	Adrenal cortex: hypertrophy (4/48, 5/48, 5/48, 4/48, 10/48); cytoplasmic vacuolization (2/48, 5/48, 5/48, 5/48, 9/48) Bone marrow: hyperplasia (0/48, 1/48, 1/48, 3/47, 4/48) Eye: retina degeneration (14/45, 16/48, 16/47, 21/45, 23/46) Ovary: atrophy (38/48, 41/48, 43/48, 44/48, 43/48) Peripheral nerve (sciatic): axon degeneration (4/48, 3/48, 1/48, 4/48, 19/48) Spleen: hematopoietic cell proliferation (8/48, 10/48, 7/48, 7/48, 15/48)	Eye: cataract (3/44, 6/44, 5/45, 6/44, 9/41) Lung: alveolar epithelium hyperplasia (0/47, 0/46, 3/47, 4/45, 9/48) Preputial gland: inflammation (3/44, 6/46, 3/47, 14/47, 15/46) Spleen: hematopoietic cell proliferation (5/45, 6/47, 9/46, 6/47, 14/45) Stomach: forestomach epithelium hyperplasia (0/46, 1/45, 3/46, 3/47, 8/44)	Eye: cataract (3/45, 2/44, 7/47, 11/45, 13/38) Ovary: cyst (8/46, 18/45, 12/48, 20/45, 18/42) Spleen: hematopoietic cell proliferation (5/46, 10/46, 6/48, 14/45, 18/44) Stomach: forestomach epithelium hyperplasia (5/46, 9/46, 4/48, 4/45, 11/42)

Summary of the 2-Year Carcinogenesis Studies of Acrylamide

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Neoplastic effects	Epididymis: malignant mesothelioma (2/48, 2/48, 1/48, 5/48, 8/48) Testes: malignant mesothelioma (1/48, 2/48, 1/48, 1/48, 5/48) Epididymis or Testes: malignant mesothelioma (2/48, 2/48, 1/48, 5/48, 8/48) Heart: malignant schwannoma (1/48, 2/48, 3/48, 4/48, 6/48) Pancreatic islets: adenoma (1/46, 2/48, 4/48, 1/48, 6/48); carcinoma (0/46, 0/48, 0/48, 1/48, 0/48); adenoma or carcinoma (1/46, 2/48, 4/48, 2/48, 6/48) Thyroid gland: follicular cell adenoma (0/47, 1/48, 1/47, 1/48, 3/48); follicular cell carcinoma (1/47, 2/48, 3/47, 6/48, 6/48); follicular cell adenoma or carcinoma (1/47, 3/48, 4/47, 6/48, 9/48)	Clitoral gland: carcinoma (1/48, 6/48, 12/47, 3/48, 8/47) Heart: malignant schwannoma (2/48,1/48, 0/48, 2/48, 4/48) Liver: hepatocellular adenoma (0/48, 0/48, 1/48, 1/48, 3/48) Mammary gland: fibroadenoma (16/48, 18/48, 24/46, 22/47, 31/48) Oral mucosa or tongue: squamous cell papilloma or carcinoma (0/48, 2/48, 1/48, 3/48, 5/48) Skin: subcutaneous tissue fibroma, fibrosarcoma, or sarcoma (1/48, 0/48, 0/48, 1/48, 5/48) Thyroid gland: follicular cell adenoma (0/48, 0/48, 1/48, 0/48, 2/47); follicular cell carcinoma (0/48, 0/48, 1/48, 3/48, 2/47); follicular cell adenoma or carcinoma (0/48, 0/48, 2/48, 3/48, 4/47)	Harderian gland: adenoma (2/46, 13/46, 27/47, 36/47, 39/47); adenocarcinoma (0/46, 0/46, 0/47, 1/47, 1/47); adenoma or adenocarcinoma (2/46, 13/46, 27/47, 37/47, 39/47) Lung: alveolar/bronchiolar adenoma (5/47, 6/46, 13/47, 10/45, 19/48); alveolar/bronchiolar adenoma or carcinoma (6/47, 6/46,14/47, 10/45, 20/48) Stomach: forestomach squamous cell papilloma (0/46, 2/45, 2/46, 6/47, 6/44); forestomach squamous cell carcinoma (0/46, 0/45, 0/46, 1/47, 2/44); forestomach squamous cell papilloma or carcinoma (0/46, 2/45, 2/46, 7/47, 8/44)	Harderian gland: adenoma (0/45, 8/44, 20/48, 32/47, 31/43) Lung: alveolar/bronchiolar adenoma (1/47, 4/47, 6/48, 11/45, 19/45) Mammary gland: adenoacanthoma (0/47, 1/46, 1/48, 2/45, 4/42); adenocarcinoma (0/47, 4/46, 6/48, 2/45, 13/42); adenoacanthoma or adenocarcinoma (0/47, 4/46, 7/48, 4/45, 17/42) Ovary: benign granulosa cell tumor (0/46, 1/45, 0/48, 1/45, 5/42) Skin: fibrosarcoma, hemangiosarcoma, liposarcoma, myxosarcoma, neurofibrosarcoma, or sarcoma (0/48, 0/46, 3/48, 10/45, 6/43) Stomach: forestomach squamous cell papilloma (4/46, 0/46, 2/48, 5/45, 8/42)
Level of evidence of carcinogenic activity	Clear evidence	Clear evidence	Clear evidence	Clear evidence

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

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

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

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

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS PEER REVIEW PANEL

The members of the Technical Reports Peer Review Panel who evaluated the draft NTP Technical Report on Acrylamide on April 5, 2011, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members have five major responsibilities in reviewing the NTP studies:

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

John Cullen, V.D.M., Ph.D., D.A.C.V.P., Chairperson
College of Veterinary Medicine
North Carolina State University
Raleigh, NC

Lucy M. Anderson, Ph.D., Consultant
Catonsville, MD

Norman J. Barlow, D.V.M., M.B.A., M.L.D., Ph.D., Primary Reviewer
Preclinical Safety
Sanofi-aventis
Bridgewater, NJ

Diane F. Birt, Ph.D., Primary Reviewer
Department of Food Science and Human Nutrition
Iowa State University
Ames, IA

Wendy J. Heiger-Bernays, Ph.D., Primary Reviewer
Department of Environmental Health
Boston University School of Public Health
Boston, MA

James E. Klaunig, Ph.D.*
Department of Environmental Health
Indiana University
Indianapolis, IN

Mark S. Miller, M.A., M.Phil., Ph.D.
School of Medicine
Wake Forest University
Winston-Salem, NC

Arlin B. Rogers, D.V.M., Ph.D.
Lineberger Comprehensive Cancer Center
University of North Carolina at Chapel Hill
Chapel Hill, NC

*Did not attend

SUMMARY OF PEER REVIEW PANEL COMMENTS

On April 5, 2011, the draft Technical Report on the toxicology and carcinogenesis studies of acrylamide received public review by the National Toxicology Program's Technical Report Peer Review Panel. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. F.A. Beland, NCTR, introduced the toxicology and carcinogenesis drinking water studies of acrylamide by describing acrylamide as a high-production chemical used in industrial processes and as a by-product in cigarette smoke and certain baked and fried starchy foods. Dr. Beland also described the design and results of the 2-week, 3-month, and 2-year acrylamide studies.

The proposed conclusions were *clear evidence of carcinogenic activity* in male and female F344/N rats and B6C3F1 mice.

Dr. Barlow, the first primary reviewer, thought the report was thorough and that the doses selected were appropriate. He felt that some of the data gaps had not been discussed adequately, leaving out essential information to understand why the study was conducted. He expressed concern that the existence of the previous bioassays on acrylamide may have introduced bias in the current study; for example, he questioned whether the slight increases in the incidences of mesothelioma in male rats seen at the highest doses were enough to derive a "clear evidence" call. He noted that the squamous cell papillomas in the male and female mice had been rated differently, although the incidences were similar, and wondered if either call should be changed. He asked for a more detailed discussion of historical control data to support the assertion that although they were older data, they were still valid for comparison. He also asked for more explanation of the staggered start of the study. He questioned the correlation in the report of Leydig cell tumors and mesothelioma, saying he was unaware of such a correlation.

Dr. Beland said the mesothelioma response was similar to the past studies, and was "a real response." He cited the large number of animals involved as the reason for the staggered start. He said he would add information to the rationale for the study in the report.

Dr. Heiger-Bernays, the second primary reviewer, requested more precision in the estimations of daily doses. She suggested attention be paid in the report to the nonneoplastic lesions that occurred in the studies, to the results from the Neuropathology Working Group, and to the involvement of the hematopoietic system. She said the report should mention the actual absorbed dose, along with the administered dose, since food is one of the major sources of exposure to acrylamide in humans. She pointed out that epigenetic mechanisms may play a role, and recommended that more attention be paid to that question in the glycidamide study.

Dr. Beland replied that water consumption was measured weekly to help determine dose, and that estimations of dose were as good as could be done in a chronic bioassay. Addressing the differences between the mice and the rats, he said that the numbers regarding induction of cancer were very similar across the two species. He explained that less attention was paid to the nonneoplastic lesions because there was so much cancer occurring in the studies. He agreed that epigenetic mechanisms were well worth looking at, and pointed out that NCTR has a large group devoted to epigenetic research.

Dr. Birt, third primary reviewer, commended Dr. Beland for his responses to the issues that had been raised. She asked that the inadequacy of the previous data stated in the study rationale be elaborated upon and clarified. She also asked for some discussion in the exposure section of occupational exposures to acrylamide. She encouraged inclusion in the discussion section of a table comparing results from these studies with the prior studies.

The panel then considered the draft report's conclusions. An addition noting that in male rats "squamous cell neoplasms of the oral cavity (mucosa or tongue) may have been related to acrylamide exposure" was proposed and discussed, and Dr. Barlow moved for its adoption. The panel voted three in favor and three opposed to the motion; as tie-breaker, Dr. Cullen voted against it, and the motion was defeated.

Dr. Rogers moved that the conclusion regarding male rats be accepted as written. Dr. Heiger-Bernays seconded. The motion carried unanimously with six votes.

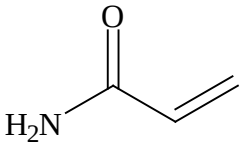
Dr. Barlow moved, and Dr. Birt seconded, that the conclusion regarding female rats be accepted as written. The motion carried unanimously with six votes.

Dr. Rogers moved, and Dr. Heiger-Bernays seconded, that the conclusion regarding male mice be accepted as written. The motion carried unanimously with six votes.

Dr. Heiger-Bernays moved, and Dr. Birt seconded, that the conclusion regarding female mice be accepted as written. The motion carried unanimously with six votes.

Dr. Rogers moved, and Dr. Birt seconded, that the conclusion regarding nonneoplastic lesions be accepted as written. The motion carried unanimously with six votes.

INTRODUCTION



ACRYLAMIDE

CAS No. 79-06-1

Chemical Formula: C_3H_5NO Molecular Weight: 71.08

Synonyms: 2-Propenamide, acrylagel, acrylic acid amide, acrylic amide, ethylenecarboxamide, propenamide, vinyl amide.

CHEMICAL AND PHYSICAL PROPERTIES

Acrylamide is an odorless, colorless-to-white, crystalline solid, with a solubility of 2.2 g per ml in water, 0.86 g per ml in ethanol, and 0.63 g per ml in acetone, and a melting point of 84° to 85° C (International Agency for Research on Cancer, 1994; Habermann, 2004).

PRODUCTION, USE, AND HUMAN EXPOSURE

Acrylamide is produced by the catalytic hydration of acrylonitrile in the presence of copper metal or Raney copper, and more recently by the enzymatic hydrolysis of acrylonitrile (International Agency for Research on Cancer, 1994; Habermann, 2004). The United States, Western Europe, and Japan account for an estimated 70% of the world capacity for acrylamide production; the annual production capacity in the United States has been reported to be 137 million kg (Habermann, 2004). The primary use of acrylamide is to produce polyacrylamides that are used in water and wastewater treatment, crude-oil, mineral, concrete, textile, and paper and pulp processing, soil and sand treatment, cosmetics, and coating applications (International Agency for Research on Cancer, 1986, 1994; Habermann, 2004; Cosmetic Ingredient Review Expert Panel, 2005).

The occupational exposure to acrylamide has been reviewed (International Agency for Research on Cancer, 1994). Permissible occupational exposure limits for acrylamide are typically 0.3 mg acrylamide per m^3 (International Agency for Research on Cancer, 1994). More recently, acrylamide has been identified as a contaminant in baked and fried carbohydrate-rich foods (*e.g.*, French fries, potato chips, bread, and cereals) (Rosén and Hellenäs, 2002; Tareke *et al*., 2002), as a consequence of Maillard reactions involving reducing sugars and asparagine, a major amino acid present in potatoes and cereals (Mottram *et al*., 2002; Stadler *et al*., 2002). In the U.S., the mean dietary exposure to acrylamide has been estimated to be 0.44 μg/kg acrylamide body weight/day for individuals over the age of 2, with the 90th percentile of exposure being approximately 0.95 μg acrylamide/kg body weight/day (Doerge *et al*., 2008). Children between the ages of 2 and 5 are estimated to be exposed to approximately twice the amount of the general population (mean, 1.1 μg acrylamide/kg body weight/day; 90th percentile, 2.3 μg acrylamide/kg body weight/day; Doerge *et al*., 2008). Similar estimates have been published for European countries (Hilbig *et al*., 2004; Boon *et al*., 2005; Dybing *et al*., 2005). Another non-occupational source of acrylamide is cigarettes, which contribute an estimated 3.1 μg acrylamide/kg body weight/day (Bergmark, 1997).

BIOLOGICAL AND TOXICOLOGICAL PROPERTIES

Absorption, distribution, metabolism, and excretion in experimental animals

The absorption, distribution, metabolism, and excretion of acrylamide in experimental animals have been reviewed (Shipp *et al*., 2006). In mice, 32% to 52% of a gavage dose is delivered into the circulation as the parent compound; a value of 23% was determined after dietary exposure (Doerge *et al*., 2005a). In rats treated by gavage, 60% to 98% of the dose of acrylamide is delivered into the circulation as the parent compound; the comparable values after dietary administration are 28% to 47% (Doerge *et al*., 2005b). Dermal application of acrylamide to rats results in approximately 20% to 30% being absorbed systemically (Sumner *et al*., 2003; Shipp *et al*., 2006).

The distribution of acrylamide after oral, intraperitoneal, intravenous, dermal, or inhalation exposure has been investigated in mice (Carlson and Weaver, 1985; Marlowe *et al*., 1986; Sumner *et al*., 2003), rats (Hashimoto and Aldridge, 1970; Miller *et al*., 1982; Ikeda *et al*., 1983; Crofton *et al*., 1996; Sumner *et al*., 2003), minipigs (Ikeda *et al*., 1983, 1985, 1987), rabbits (Ikeda *et al*., 1983), dogs (Ikeda *et al*., 1983, 1985, 1987), and trout (Waddell *et al*., 1990). In each instance, acrylamide was rapidly distributed to all tissues investigated, including the fetuses of pregnant animals.

Acrylamide is converted to a reactive epoxide metabolite, glycidamide (Calleman *et al*., 1990), primarily through the action of cytochrome P450 2E1 (Sumner *et al*., 1999; Ghanayem *et al*., 2005a). At a dose of 50 mg acrylamide per kg body weight, mice produce quantitatively more glycidamide than do rats (Sumner *et al*., 1992). In rats, the formation of glycidamide is linear at low doses of acrylamide, but saturation of enzymatic oxidation occurs at high doses (Bergmark *et al*., 1991). At low doses of acrylamide (*e.g.*, 5 mg acrylamide per kg body weight), rats convert more than 50% of the acrylamide to glycidamide; the extent of conversion decreases at higher doses (*e.g.*, 13% conversion at 100 mg acrylamide per kg body weight) (Bergmark *et al*., 1991).

After oral gavage of acrylamide to mice, the elimination half-life ($t_{1/2}$) of glycidamide (0.7-1.5 hr) is similar to that of acrylamide (1.3-1.9 hr) and the ratio of internal exposure ($AUC_{0-\infty}$) of glycidamide:acrylamide is 1.0-2.9 (Twaddle *et al*., 2004a; Doerge *et al*., 2005a). In rats treated orally with acrylamide, the $t_{1/2}$ of glycidamide is 1.9-2.6 hr, the $t_{1/2}$ of acrylamide is 1.6-2.2 hr, and $AUC_{0-\infty}$ ratio of glycidamide:acrylamide is 0.22-0.96 (Barber *et al*., 2001; Doerge *et al*., 2005b). The higher ratio of glycidamide:acrylamide observed in mice compared to rats is consistent with a more efficient conversion of acrylamide to glycidamide in mice, particularly at high doses of acrylamide.

In mice and rats, acrylamide can be directly conjugated with glutathione, which is detected in the urine as *S*-(2-carbamoylethyl)cysteine and *N*-acetyl-*S*-(2-carbamoylethyl)cysteine (Figure 1; Sumner *et al*., 1992, 1999, 2003; Doerge *et al*., 2007; Kopp and Dekant, 2009). Glycidamide can also be conjugated with glutathione, which yields *N*-acetyl-*S*-(1-carbamoyl-2-hydroxyethyl)-cysteine and *N*-acetyl-*S*-(2-carbamoyl-2-hydroxyethyl)-cysteine as urinary metabolites (Figure 1; Sumner *et al*., 1992, 1999, 2003; Doerge *et al*., 2007; Kopp and Dekant, 2009). Glycidamide also undergoes hydrolysis to give 2,3-dihydroxypropanamide (glyceramide) and subsequently 2,3-dihydroxypropionic acid (Figure 1; Sumner *et al*., 1992, 1999, 2003).

Acrylamide reacts slowly with DNA to give a number of DNA adducts *in vitro*. These are (listed in order of decreasing yield) N1-(2-carboxyethyl)-deoxyadenosine, N3-(2-carboxyethyl)deoxycytidine, N7-(2-carbamoylethyl)guanine (from the depurination of N7-(2-carbamoylethyl)deoxyguanosine), N^6-(2-carboxyethyl)deoxyadenosine, and N1-(2-carboxy-ethyl)deoxyguanosine (Figure 2; Solomon *et al*., 1985). When reactions are conducted with deoxynucleosides, N3-(2-carbamoylethyl)thymidine, N7,9-(*bis*-2-carbamoylethyl)guanine (from further reaction with N7-(2-carbamoylethyl)guanine), and imidazole ring-opened N7,9-(*bis*-2-carbamoylethyl)guanine also result (Figure 2; Solomon *et al*., 1985). To date, these DNA adducts have not been detected in experimental animals.

Glycidamide is considerably more reactive than acrylamide with DNA *in vitro* and several adducts have been characterized, including N7-(2-carbamoyl-2-hydroxyethyl)guanine (N7-GA-Gua; from the depurination of N7-(2-carbamoyl-2-hydroxy-ethyl)deoxyguanosine), N3-(2-carbamoyl-2-hydroxy-ethyl)adenine (N3-GA-Ade; from the depurination of N3-(2-carbamoyl-2-hydroxyethyl)deoxyadenosine), N1-(2-carboxy-2-hydroxyethyl)deoxyadenosine, N^6-(2-carboxy-2-hydroxyethyl)deoxyadenosine (from a Dimroth rearrangement of N1-(2-carboxy-2-hydroxy-ethyl)deoxyadenosine), N1,N^6-(2-hydroxypropanoyl)-deoxyadenosine, and N3,N^4-(2-hydroxypropanoyl)-deoxycytidine (Figure 3; Segerbäck *et al*., 1995; Solomon, 1999; Gamboa da Costa *et al*., 2003).

N7-GA-Gua and N3-GA-Ade have been detected in mice and rats treated with acrylamide (Segerbäck *et al.*,

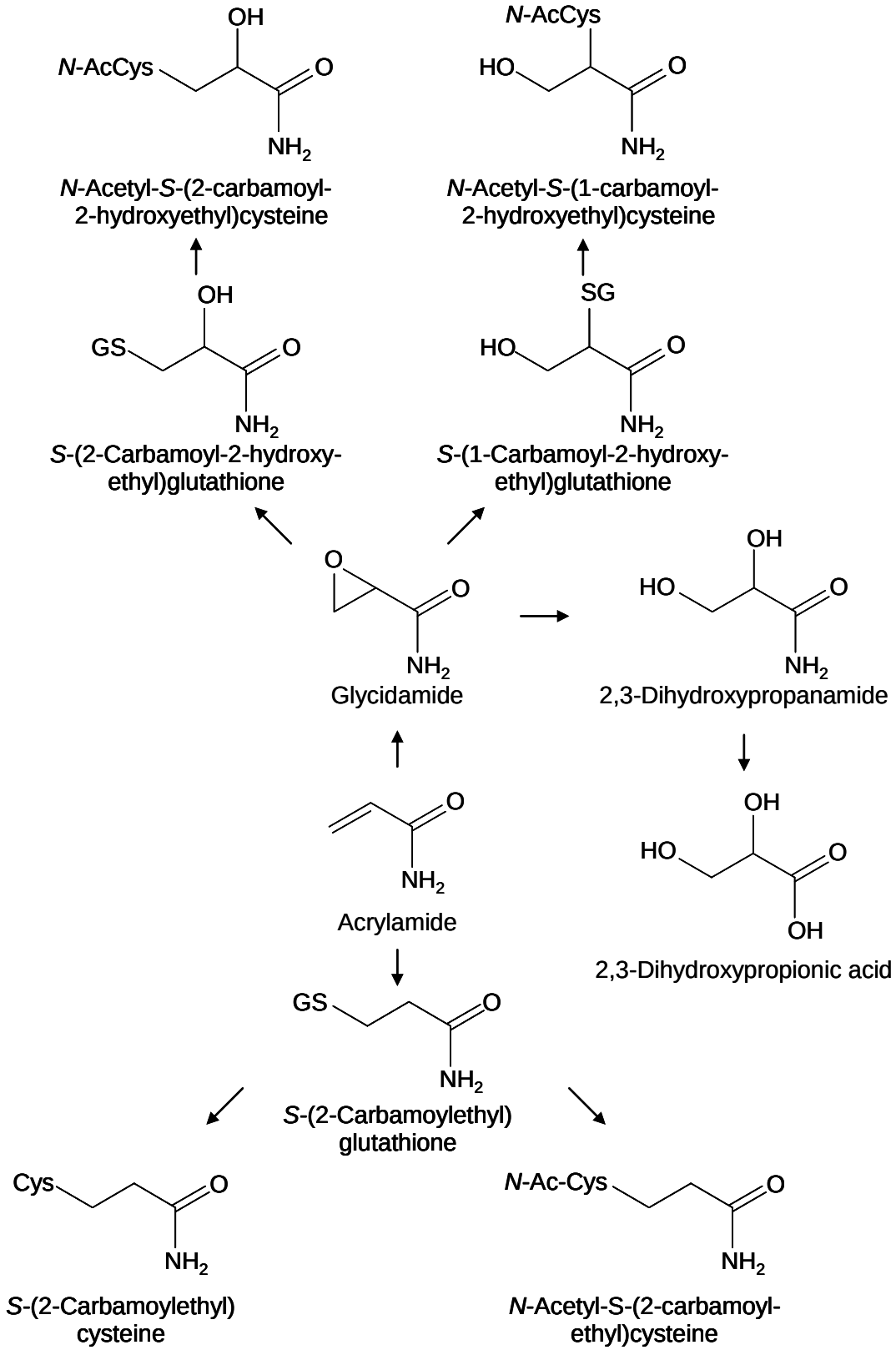


FIGURE 1
Metabolites of Acrylamide

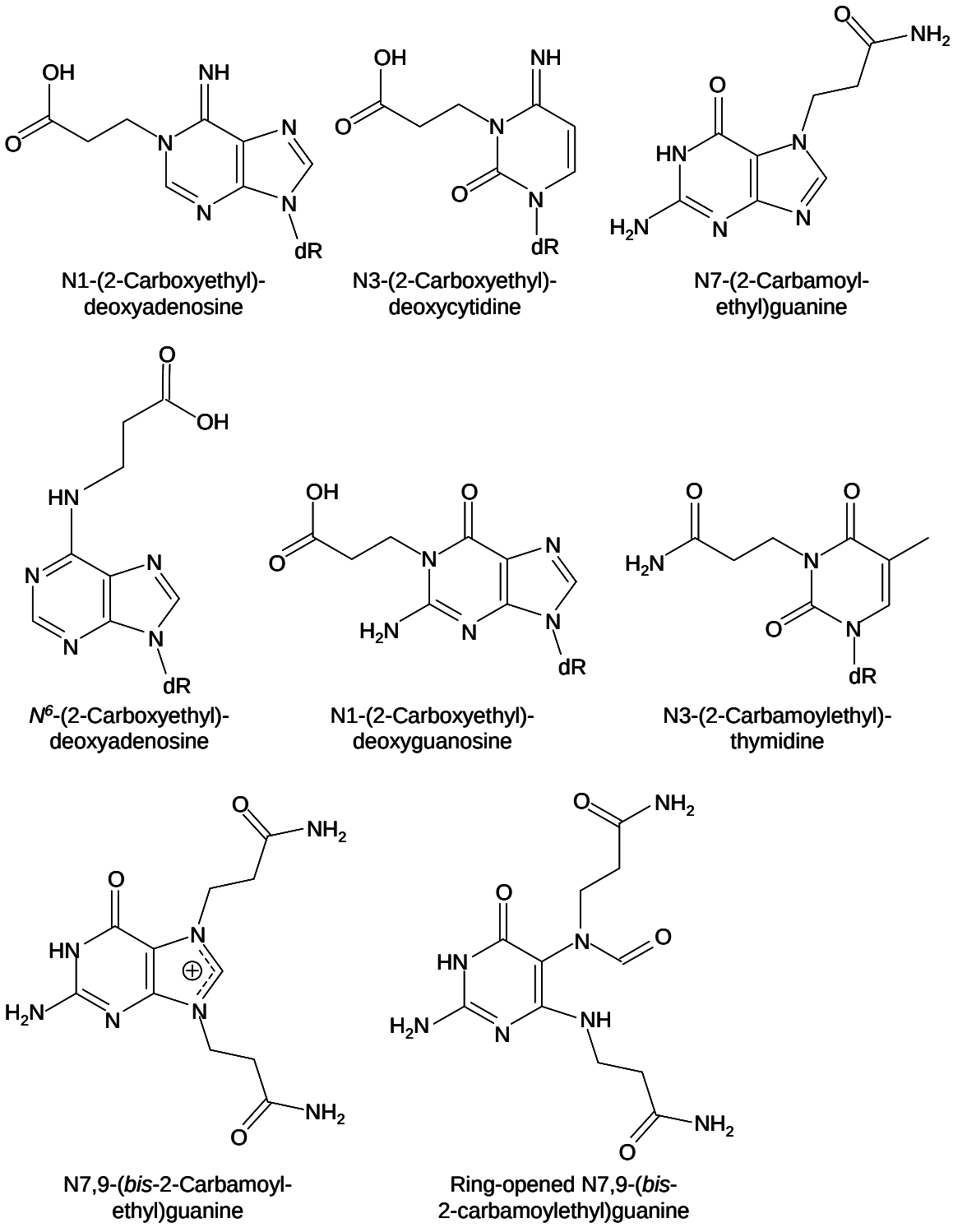
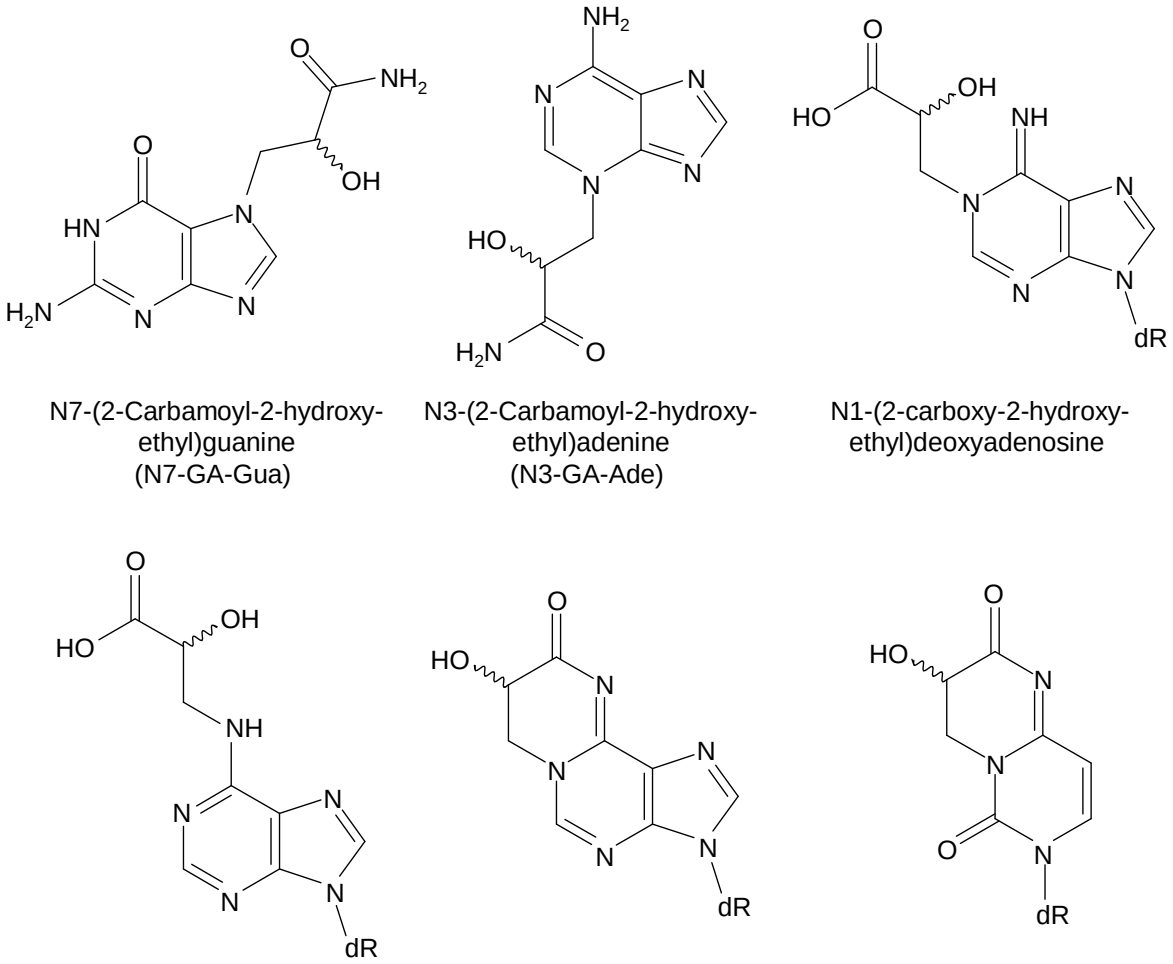


FIGURE 2
DNA Adducts of Acrylamide



N7-(2-Carbamoyl-2-hydroxy-ethyl)guanine (N7-GA-Gua)

N3-(2-Carbamoyl-2-hydroxy-ethyl)adenine (N3-GA-Ade)

N1-(2-carboxy-2-hydroxy-ethyl)deoxyadenosine

N^6-(2-carboxy-2-hydroxy-ethyl)deoxyadenosine

N1,*N*^{6}-(2-hydroxypropanoyl)-deoxyadenosine

N3,*N*^{4}-(2-hydroxypropanoyl)-deoxycytidine

FIGURE 3
DNA Adducts of Glycidamide

1995; Gamboa da Costa *et al*., 2003; Twaddle *et al*., 2004a; Doerge *et al*., 2005a,b,c; Ghanayem *et al*., 2005a; Manière *et al*., 2005; Tareke *et al*., 2006; Von Tungeln *et al*., 2009; Zeiger *et al*., 2009). Typically, N7-GA-Gua is formed to a 100-fold greater extent than N3-GA-Ade (Gamboa da Costa *et al*., 2003; Ghanayem *et al*., 2005a; Manière *et al*., 2005; Tareke *et al*., 2006; Von Tungeln *et al*., 2009), a ratio that corresponds to that observed in DNA reacted with glycidamide *in vitro* (Gamboa da Costa *et al*., 2003). Both adducts are normally detected in all tissues examined, including tissues susceptible to tumor formation (Segerbäck *et al.*, 1995; Gamboa da Costa *et al*., 2003; Doerge *et al*., 2005c; Ghanayem *et al*., 2005a; Manière *et al*., 2005**;** Tareke *et al*., 2006; Von Tungeln *et al*., 2009). At high doses of acrylamide, the levels of adducts from acrylamide tend to be higher in mice than in rats, which is consistent with the more extensive conversion of acrylamide to glycidamide in mice as compared to rats (Doerge *et al*., 2005c). In mice, hepatic levels of N7-GA Gua increase in a linear manner with dose (Zeiger *et al*., 2009).

The $t_{1/2}$ values for the loss (removal) of N7-GA-Gua from DNA in rats after a single dose of acrylamide are 19-89 hours, depending upon the tissue; the $t_{1/2}$ values for N3-GA-Ade are 19-33 hours (Manière *et al*., 2005). Since these values are similar to those observed *in vitro* (Gamboa da Costa *et al*., 2003), this suggests the decrease in adduct levels *in vivo* is due to spontaneous depurination rather than active DNA repair processes (Doerge *et al*., 2005c). After continuous administration of acrylamide to rats, the loss of N7-GA-Gua tends to be slower than after a single treatment (Tareke *et al*., 2006).

Acrylamide and its oxidation product glycidamide react with cysteine residues in hemoglobin and other proteins (Bergmark *et al*., 1991). After hydrolysis with 6 N HCl, the products are released as *S*-(2-carboxyethyl)cysteine (from acrylamide) and *S*-(2-carboxy-2-hydroxyethyl)-cysteine (from glycidamide) (Figure 4). Acrylamide and glycidamide also react with the N-terminal valine of hemoglobin to give (after acid hydrolysis) *N*-(2-carboxyethyl)valine (from acrylamide) and *N*-(2-carboxy-2-hydroxyethyl)valine (from glycidamide) (Figure 4; Bergmark *et al*., 1993).

The ratio of acrylamide to glycidamide protein adducts is dependent on dose and species. In mice, glycidamide-hemoglobin adducts are formed to a greater extent than acrylamide-hemoglobin adducts at all doses investigated, regardless of the route of administration (Paulsson *et al*., 2002; Tareke *et al*., 2006; Zeiger *et al*., 2009), which probably reflects the more extensive oxidation of acrylamide in mice, and 2004a; Doerge *et al*., 2005a,b,c; Ghanayem *et al*., there is a linear relationship between the administered dose of acrylamide and the concentration of glycidamide-hemoglobin adducts (Zeiger *et al*., 2009). In rats administered a single intraperitoneal dose of acrylamide (0 to 100 mg per kg body weight), the formation of acrylamide-cysteine adducts [*S*-(2-carboxyethyl)cysteine] in hemoglobin is linear, whereas the formation of glycidamide-cysteine adducts [*S*-(2-carboxy-2-hydroxyethyl)cysteine] plateaus at high doses, which suggests metabolic saturation (Bergmark *et al*., 1991). As a consequence, at high doses of acrylamide (*e.g*., 100 mg per kg body weight administered intraperitoneally) in rats, the concentration of acrylamide-hemoglobin adducts exceeds the concentration of glycidamide-hemoglobin adducts, whether measured as cysteine or N-terminal valine adducts (Bergmark *et al*., 1991; Paulsson *et al*., 2002). As the dose of acrylamide is lowered to 3 mg acrylamide per kg body weight (administered by gavage), the ratio of acrylamide-hemoglobin adducts to glycidamide-hemoglobin adducts, as measured by N-terminal valine adducts, approaches unity (Fennell *et al*., 2005), while at even lower doses (100 µg acrylamide per kg body weight given orally or intravenously), glycidamide-hemoglobin adducts predominate over acrylamide-hemoglobin adducts (Tareke *et al*., 2006). At doses of 100 µg acrylamide per kg body weight, the levels of glycidamide-hemoglobin adducts in rats can exceed those in mice (Tareke *et al*., 2006), and in both mice and rats, there is a linear relationship between the concentration of glycidamide-hemoglobin adducts and hepatic levels of N7-GA-Gua (Tareke *et al*., 2006; Zeiger *et al*., 2009).

Absorption, Distribution, Metabolism, and Excretion in Humans

The absorption, distribution, metabolism, and excretion of acrylamide in humans have been reviewed (Shipp *et al*., 2006).

In humans treated orally, acrylamide has a serum t_{max} of 0.94 hr and a $t_{1/2}$ of 0.79 hr (Kopp and Dekant, 2009), and 34-71% of the administered dose is excreted in the urine (Fennell *et al*., 2005, 2006; Boettcher and Angerer, 2005; Boettcher *et al*., 2006; Fuhr *et al*., 2006; Hartmann *et al*., 2009; Kopp and Dekant, 2009). Among the urinary metabolites that have been identified are acrylamide, *N*-acetyl-*S*-(2-carbamoylethyl)cysteine, glycidamide, 2,3-dihydroxypropanamide, *N*-acetyl-*S*-(1-carbamoyl-2-hydroxyethyl)cysteine, and *N*-acetyl-*S*-(2-carbamoyl-2-hydroxyethyl)cysteine (Figure 1; Fennell *et al*., 2005, 2006; Boettcher and Angerer, 2005;

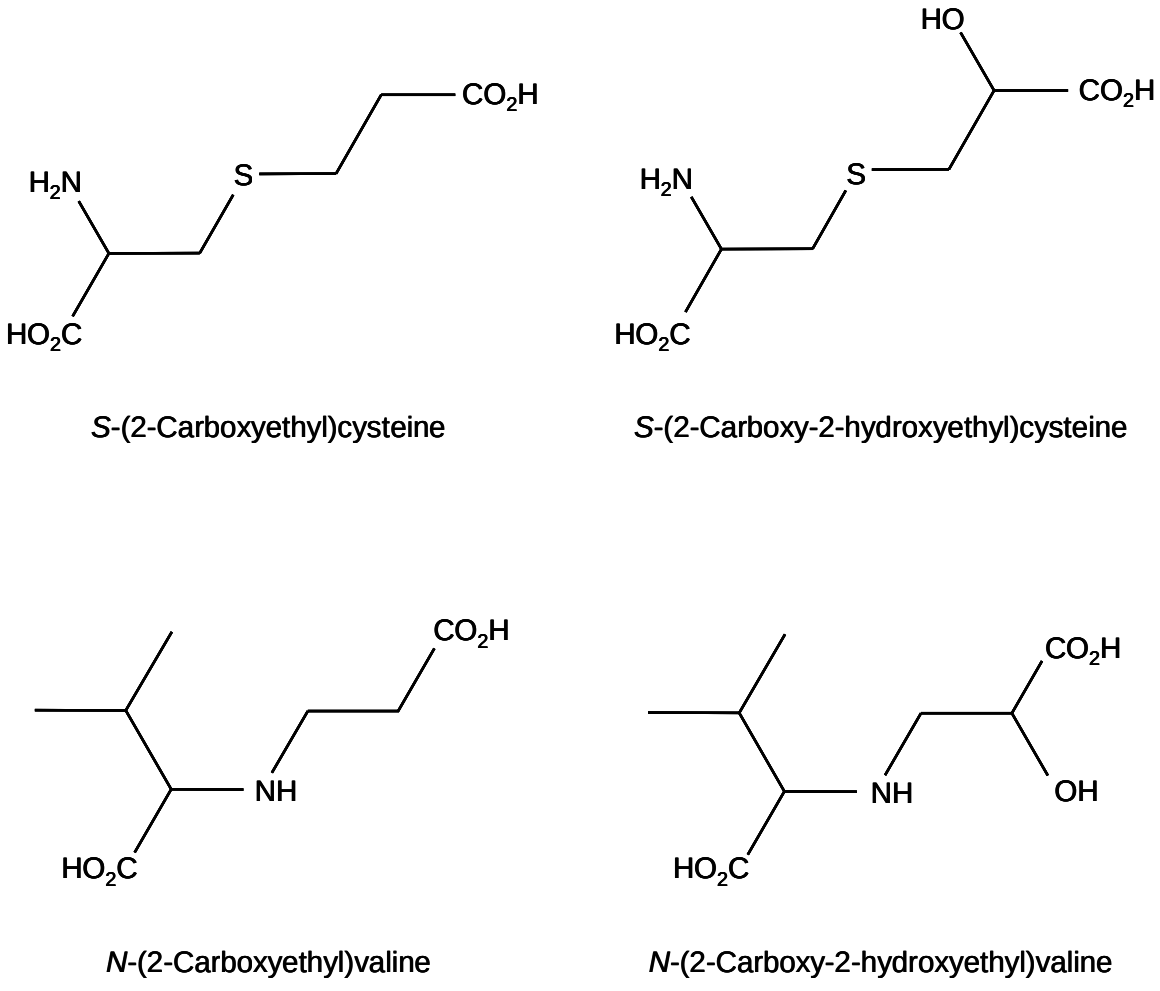


FIGURE 4
Cysteine and Valine Adducts of Acrylamide and Glycidamide

Boettcher *et al*., 2006; Fuhr *et al*., 2006; Hartmann *et al*., 2009; Kopp and Dekant, 2009). In addition to these metabolites, *N*-acetyl-*S*-(2-carbamoylethyl)-cysteine *S*-sulfoxide, a metabolite not detected in rodent urine, has been identified in humans. Metabolites arising from acrylamide (*i.e*., *N*-acetyl-*S*-(2-carbamoyl-ethyl)cysteine and its sulfoxide) account for the majority (greater than 60%) of the urinary excretion, and the ratio of glycidamide-derived urinary metabolites to acrylamide-derived urinary metabolites is 0.02-0.16 (Fennell *et al*., 2005, 2006; Boettcher and Angerer, 2005; Boettcher *et al*., 2006; Fuhr *et al*., 2006; Doroshyenko *et al*., 2009; Hartmann *et al*., 2009; Kopp and Dekant, 2009). This low ratio of glycidamide-derived urinary metabolites to acrylamide-derived urinary metabolites has been interpreted as evidence that the conversion of acrylamide to glycidamide occurs to a lesser extent in humans as compared to rodents; however, pharmacokinetic modeling results have indicated only minor species differences in the metabolism of acrylamide to glycidamide at low doses of acrylamide (Walker *et al*., 2007; Young *et al*., 2007; Sweeney *et al*., 2010).

In humans, acrylamide and its oxidation product glycidamide react with cysteine and the N-terminal valine residues in hemoglobin, and measurements of the N-terminal valine adducts have been used extensively to monitor occupational exposure to acrylamide (reviewed in Shipp *et al*., 2006). The formation of acrylamide and glycidamide hemoglobin adducts has also been measured after oral administration of acrylamide to humans, and the ratio of glycidamide valine adducts to acrylamide valine adducts is 0.4. This low ratio has been suggested as being due to limited oxidation (compared to rodents) of acrylamide to glycidamide (Fennell *et al*., 2005) but it could also be due to deficiencies in the analytical methodology for measuring glycidamide valine adducts as compared to acrylamide valine adducts. The formation of DNA adducts from acrylamide in humans has not been reported, although recent physiologically based pharmacokinetic/pharmacodynamic modeling suggests that the dietary exposure to acrylamide should result in N7-GA-Gua levels of 0.06-0.5 adducts per 10^8 nucleotides (Doerge *et al*., 2008; Young *et al*., 2007).

Toxicity in Experimental Animals

Acrylamide is a neurotoxin in experimental animals. This has been demonstrated in mice, rats, guinea pigs, rabbits, cats, dogs, and monkeys and has been the subject of a number of reviews (International Agency for Research on Cancer, 1986, 1994; LoPachin, 2005; Exon, 2006; Shipp *et al*., 2006). The major overt neurotoxic response is loss of motor function, as exemplified by hind-limb splay and impaired rotorod performance. The overt response is accompanied by biochemical changes, as well as ultrastructural alterations that can be observed microscopically.

Subchronic oral administration of acrylamide to rats results in loss of motor function at doses of greater than or equal to 9 mg acrylamide per kg body weight per day (Shipp *et al*., 2006). Loss of motor function occurs at a similar dose level in mice and monkeys. Somewhat higher doses are required to elicit the same response in cats (15 mg acrylamide per kg body weight per day), while in dogs, the loss of motor function occurs at lower doses (6 to 7 mg acrylamide per kg body weight per day).

Toxicity in Humans

In humans, acrylamide is a skin and respiratory irritant and a neurotoxin (reviewed in International Agency for Research on Cancer, 1986, 1994; Shipp *et al*., 2006). The neurological signs of toxicity include numbness of hands and feet and impairment of sensation. In severe cases, there can be loss of reflexes, muscular atrophy, body weight decreases, and ataxia.

Reproductive Toxicity and Teratogenicity in Experimental Animals

Acrylamide is a reproductive toxicant in experimental animals (reviewed in International Agency for Research on Cancer, 1986, 1994; Exon, 2006; Shipp *et al*., 2006). In mice and rats, acrylamide administered orally at doses of greater than or equal to 5 mg acrylamide per kg body weight per day causes increases in post-implantation loss, which results in a decrease in the number of live pups per litter. Higher doses cause neurotoxicity, changes in breeding behavior, and effects on sperm motility and morphology.

At doses of greater than or equal to 15 mg per kg body weight per day, acrylamide does not affect embryo/fetal viability, growth, or development in mice and rats. Higher doses, which are associated with maternal neurotoxicity, result in reduced pup weight and survival.

Acrylamide administration causes significant decreases in fertility, increases in dominant lethality, and increases in heritable translocations in mice and/or rats.

Reproductive Toxicity and Teratogenicity in Humans

The reproductive and developmental effects of acrylamide in humans have recently been reviewed

(NTP-CERHR Monograph, 2005). There is no evidence for adverse reproductive or developmental effects from exposure to acrylamide in the general population, and, while occupational exposure to acrylamide can be associated with neurotoxicity, it is currently not known if reproductive and/or developmental toxicity will also occur.

Carcinogenicity in Experimental Animals

The carcinogenicity of acrylamide has been assessed in mice and rats. These experiments are summarized in the following paragraphs.

Male and female A/J mice (40 mice per sex per treatment) were administered acrylamide by gavage three times per week for 8 weeks at doses of 0, 6.25, 12.5, and 25.0 mg acrylamide per kg body weight per treatment. Acrylamide caused a significant dose-related increase in the incidence and multiplicity of lung adenoma when assessed at 7 months after the initiation of treatment (Bull *et al*., 1984a). In a second experiment, male and female A/J mice (15 to 17 mice per sex per treatment) were given intraperitoneal injections of acrylamide three times per week for 8 weeks at doses of 0, 1, 3, 10, and 30 mg acrylamide per kg body weight per treatment. A treatment of 60 mg acrylamide per kg body weight was also attempted but was discontinued due to the onset of neurotoxicity (frank peripheral neuropathy). When assessed 6 months after the initiation of treatment, acrylamide caused a significant dose-related increase in the incidence and multiplicity of lung adenoma (Bull *et al*., 1984a).

Female SENCAR mice (40 mice per group) were dosed six times orally, intraperitoneally, or topically with 0, 12.5, 25.0, or 50.0 mg acrylamide per kg body weight over a 2 week period. Two weeks after the last dose, the mice were treated topically with 1.0 µg 12-*O*-tetradecanoylphorbol-13-acetate (TPA) three times per week for 20 weeks. Additional mice (20 mice per group) were administered the high dose of acrylamide but not given TPA. Fifty-two weeks after the initiation of the study, mice treated with acrylamide and TPA had a dose-related increase in skin squamous cell papilloma and carcinoma irrespective of the route of administration. Skin tumors did not occur in the absence of TPA treatment (Bull *et al*., 1984a).

A subsequent study consisted of two experiments. In the first experiment, female SENCAR mice (60 per group) were given a single intraperitoneal injection of 0 or 50 mg acrylamide per kg body weight. Two weeks later, 40 mice from each group were treated topically with 1.0 µg TPA three times per week for 20 weeks. The mice were monitored for 1 year after the initial treatment, at which time the mice given acrylamide and TPA had a significant increase in the multiplicity of skin papilloma. In the second experiment, female SENCAR, BALB/c, A/J, and ICR mice (60 per group) were treated in a manner identical to the first experiment, with the doses of TPA being 1.0 µg for SENCAR mice, 5.0 µg for BALB/c mice, and 2.5 µg for A/J and ICR mice. One year after the initial treatment, SENCAR mice administered acrylamide and TPA had a significant increase in the multiplicity of skin papilloma and lung adenoma. There was not an increase in tumorigenicity in the other strains of mice (Robinson *et al*., 1986).

Female Swiss-ICR mice (40 per group) were dosed orally, intraperitoneally, or topically with 0, 12.5, 25.0, or 50.0 mg acrylamide per kg body weight six times over a 2 week period. Two weeks after the last dose, the mice were treated topically with 2.5 µg TPA three times per week for 20 weeks. An additional group of 40 mice was administered the high dose of acrylamide but was not treated with TPA. When assessed 52 weeks after the initiation of the study, mice given 50 mg acrylamide per kg body weight and TPA had a significant increase in the multiplicity of skin tumors (squamous cell papilloma and carcinoma). There was also a significant increase in the incidence of alveolar/bronchiolar adenoma or carcinoma in the mice administered the high dose of acrylamide, irrespective of TPA treatment (Bull *et al*., 1984b).

Male and female Fischer 344 (F344) rats (60 per sex per group) were given 0, 0.01, 0.2, 0.5, or 2.0 mg acrylamide per kg body weight per day in the drinking water for 2 years. Male rats receiving 2.0 mg acrylamide per kg body weight had a significant increase in thyroid gland adenoma and mesothelioma of the tunica vaginalis of the testes; an increase in the mesotheliomas also occurred at 0.5 mg acrylamide per kg body weight. Female rats receiving 2.0 mg acrylamide per kg body weight had significant increases in benign mammary gland (adenoma, fibroadenoma, or fibroma) tumors, central nervous system (CNS) tumors, and thyroid gland adenoma or adenocarcinoma. A non-significant increase in CNS tumors was also observed in male rats (Johnson *et al*., 1986).

In a subsequent study, male F344 rats were administered 0.1, 0.5, or 2.0 mg acrylamide per kg body weight in the drinking water for 2 years (204, 102, and 75 rats, respectively). Two additional groups consisting of 102 male rats per group served as controls. Female F344 rats (100 rats per group) were given 1.0 or 3.0 mg acrylamide per kg body weight in the drinking water for 2 years, with two additional groups of 50 female rats per group serving as controls. In male rats treated with

2.0 mg acrylamide per kg body weight, there was a significant increase in thyroid gland adenoma and mesothelioma of the tunica vaginalis of the testes. In female rats given 1.0 or 3.0 mg acrylamide per kg body weight, there was an increase in mammary gland fibroadenoma, combined mammary gland fibroadenoma or adenocarcinoma, and combined thyroid gland follicular cell adenoma or carcinoma (Friedman *et al*., 1995). In contrast to the previous study with F344 rats that demonstrated an increased incidence of CNS tumors in females, there was not a significant increase in CNS tumors in either sex.

Carcinogenicity in Humans

The carcinogenicity of acrylamide in humans after occupational or dietary exposure has been reviewed (International Agency for Research on Cancer, 1994; Erdreich and Friedman, 2004; Rice, 2005; Shipp et al., 2006; Mucci and Wilson, 2008; Mucci and Adami, 2009). In individuals exposed occupationally to acrylamide, there has been no consistent dose-related increase in cancer incidence at any organ site, with the possible exception of pancreas. Dietary exposure to acrylamide has not been associated with an increased risk of colorectal, bladder, esophageal, prostate, oropharyngeal, laryngeal, pancreatic, gastric, or lung cancer. Data regarding the effect of dietary acrylamide on the risk of breast, renal, ovarian, and endometrial cancer are inconsistent. Subsequent to these reviews, additional epidemiological studies have appeared that examined the relationship between dietary acrylamide and brain, breast, endometrial, head and neck, ovarian, prostate, and thyroid cancer (Hogervorst *et al*., 2009; Larsson *et al*., 2009a,b; Schouten *et al*., 2009; Wilson *et al*., 2010). All were negative, with the exception of ovarian cancer, which was positive (Wilson *et al*., 2010), and endometrial cancer, for which both positive (Wilson *et al*., 2010) and negative (Larsson *et al*., 2009b) results were reported.

Genetic Toxicity

Bacterial mutagenesis assays

Acrylamide is not mutagenic in *Salmonella typhimurium* tester strains TA97, TA98, TA100, TA102, TA1535, TA1537, and TA1538, either in the presence or absence of an exogenous metabolic system (reviewed in International Agency for Research on Cancer, 1994; Dearfield *et al*., 1995; Shipp *et al*., 2006; Besaratinia and Pfeifer, 2007). Acrylamide is also not mutagenic in a reverse mutation assay with *Escherichia coli* or a forward mutation assay with *Klebsiella pneumoniae*, but induces differential toxicity in a *Bacillus subtilis rec* assay (reviewed in International Agency for Research on Cancer, 1994; Dearfield *et al*., 1995; Shipp *et al*., 2006).

In vitro mammalian gene mutation assays

Acrylamide is weakly mutagenic in L5178Y/*Tk*$^{+/-}$ mouse lymphoma cells and Big Blue mouse embryonic fibroblasts, but not mutagenic in Chinese hamster V79 cells (reviewed in International Agency for Research on Cancer, 1994; Dearfield *et al*., 1995; Shipp *et al*., 2006; Besaratinia and Pfeifer, 2007; also see Mei *et al*., 2008). The increase in mutation frequency in the Big Blue mouse embryonic fibroblasts was associated with an increase in A→ G transition and G → C transversion mutations (Besaratinia and Pfeifer, 2003, 2004).

In vivo mammalian gene mutation assays

Acrylamide gave a positive mutagenic response in the mouse spot test assay (Neuhäuser-Klaus and Schmahl, 1989). When assessed in the *lacZ* gene of transgenic Muta mice, acrylamide was not mutagenic in liver (Krebs and Favor, 1997) but gave an increased mutant frequency in bone marrow (Hoorn *et al*., 1993), although the latter response was considered to be equivocal (International Agency for Research on Cancer, 1994; Dearfield *et al*., 1995).

Transgenic Big Blue mice given acrylamide in the drinking water had increased mutant frequencies at the endogeneous *Hprt* gene in spleen T-lymphocytes and at the exogeneous *cII* gene in liver, lung, and testes (Manjanatha *et al*., 2006; Guo *et al*., 2009; Wang *et al*., 2010). Molecular analysis of the *cII* mutations from liver tissue indicated G→ T transversion mutations and -1 and +1 frameshift mutations in runs of G's (Manjanatha *et al*., 2006). Transgenic Big Blue rats administered acrylamide in the drinking water had an increased mutant frequency at the endogeneous *Hprt* gene in spleen T-lymphocytes of both sexes and at the exogeneous *cII* gene in the thyroid and bone marrow of females (Mei *et al*., 2010).

Neonatal B6C3F1/*Tk*$^{+/-}$ mice treated on postnatal days 1, 8, and 15 with acrylamide did not have an increased mutant frequency at either the *Hprt* or *Tk* gene of spleen T-lymphocytes (Von Tungeln *et al*., 2009). In contrast, treatment on postnatal days 1-8 resulted in an increased mutant frequency at both genes, with the *Tk* mutations being associated with loss of heterozygosity (Von Tungeln *et al*., 2009).

Chromosomal aberrations, sister chromatid exchange, unscheduled DNA synthesis, and cell transformation

The ability of acrylamide to induce chromosomal aberrations, sister chromatid exchange, unscheduled DNA synthesis, and cell transformation has been reviewed extensively (International Agency for

Research on Cancer, 1994; Dearfield *et al*., 1995; Shipp *et al*., 2006). Briefly, acrylamide causes chromosomal aberrations *in vitro* and *in vivo*, including in cultured human lymphocytes, induces sister chromatid exchange *in vitro* and *in vivo*, produces equivocal or marginal increases in unscheduled DNA synthesis, and has the ability to transform rodent cell lines.

STUDY RATIONALE

Acrylamide has been detected in certain baked and fried starchy foods. Existing data indicated that acrylamide is carcinogenic; however, the Center for Food Safety and Applied Nutrition, FDA, desired a modern, more definitive bioassay to perform a quantitative risk assessment. Consequently the FDA nominated acrylamide for evaluation by the NTP. Acrylamide was hypothesized to be a genotoxic carcinogen as a result of metabolic conversion to glycidamide, which reacts with DNA. Since the metabolic conversion occurs to a greater extent in mice as compared to rats, mice were hypothesized to be more sensitive than rats to the carcinogenic effects of acrylamide. To test these hypotheses and to provide data for a meaningful risk assessment, studies were conducted to compare the extent and types of tumors in B6C3F1 mice and F344/N rats treated chronically with either acrylamide or glycidamide. The data from the animals exposed to acrylamide form the basis of this report. The results from the studies with glycidamide will form the basis of a subsequent report.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

Acrylamide was purchased as a single lot (Lot 102K0162) from Sigma Chemical Co., St. Louis, MO. The identity and purity of the chemical was assessed at the National Center for Toxicological Research (NCTR) by gas chromatography coupled with electron impact mass spectrometry (GC/EI-MS), nuclear magnetic resonance (NMR) spectrometry, and gas chromatography using flame ionization detection (GC-FID).

GC/EI-MS of the acrylamide indicated a major component, with the proper mass (*m/z* = 71), that accounted for 99.4% of the material. ^{1}H and ^{13}C NMR spectra were consistent with the structure of acrylamide, and based upon the ^{1}H NMR spectra, the purity was estimated to be greater than 99.9%. GC-FID of the acrylamide indicated a major peak that accounted for greater than 99.9% of the material.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

Drinking Water

The stability of acrylamide in drinking water was assessed at a concentration of 10 µg/ml for a period of 49 days at room temperature in the absence of light. During this period, the recovery of acrylamide varied between 90.1% and 106%.

For the 2-week study, acrylamide drinking water solutions were prepared weekly for treating animals. The target acrylamide concentrations were 10 µg/ml (0.14 mM), 25 µg/ml (0.35 mM), 50 µg/ml (0.70 mM), 100 µg/ml (1.41 mM), 250 µg/ml (3.52 mM), and 500 µg/ml (7.03 mM). Concentrations were deemed acceptable if they were within 10% of the target concentrations; for the 10 µg/ml concentration, 20% of the target concentration was considered acceptable. Dose certification analyses were conducted on all acrylamide drinking water solutions and, with the exception of the 25 µg/ml sample from week 2, which was 89% of the target concentration, each met the indicated specifications (Table F2).

For the 3-month study, drinking water solutions were prepared at 2 to 3 week intervals beginning on July 15, 2004, and ending on October 1, 2004. The target acrylamide concentrations were 10 µg/ml (0.14 mM), 25 µg/ml (0.35 mM), 50 µg/ml (0.70 mM), 100 µg/ml (1.41 mM), and 250 µg/ml (3.52 mM). Concentrations were deemed acceptable if they were within 10% of the target concentrations; for the 10 µg/ml concentration, 20% of the target concentration was considered acceptable. Dose certification analyses were conducted on all acrylamide drinking water solutions and each met the indicated specifications (Table F3).

For the 2-year study, acrylamide drinking water solutions for treating the animals were prepared weekly, beginning on May 24, 2005, and ending on August 14, 2007. The target acrylamide concentrations were 6.25 µg/ml (0.0875 mM), 12.5 µg/ml (0.175 mM), 25 µg/ml (0.35 mM), and 50 µg/ml (0.70 mM). Concentrations were deemed acceptable if they were within 10% of the target concentrations; for the 6.25 µg/ml concentration, 20% of the target concentration was considered acceptable. Dose certification analyses were conducted at approximately bi-monthly intervals (Table F4). Each of the assayed acrylamide drinking water solutions met the indicated specifications. Acrylamide was not detected in the control drinking water solutions (limit of quantitation was 2 µg/ml).

Feed

Purina 5LG6 diet (also referred to as NIH-31 IR) was selected for the study because it has a very low acrylamide content (less than 50 ppb; Table H4) compared to other commercial formulations (Twaddle *et al*., 2004b).

The homogeneity of acrylamide in the diet was assessed at a concentration of 37 µg/g. The results for nine replicate samples were 33.3 ± 3.3 µg/g (mean ± s.d; range = 29.3-39.6 µg/g). The stability of acrylamide in the diet was assessed at a concentration of 37 µg/g for

a period of 28 days at room temperature and 42 days at 2° to 8° C. At room temperature, the recovery of acrylamide varied between 87.9% and 112% for a period of 21 days. By 28 days, the recovery decreased to 65.2%. At 2° to 8° C, the recovery of acrylamide varied between 90.1% and 117% for a period of 28 days. By 42 days, the recovery decreased to 62.9%.

For the 2-week study, acrylamide feed was prepared one time for treating the animals. The target concentrations were 7.4, 18.5, 37.0, 74.0, 185, and 370 mg/kg. Concentrations were deemed acceptable if they were within 10% of the target concentrations; for the 7.4 mg/kg concentration, 20% of the target concentration was considered acceptable. Dose certification analyses were conducted on all samples. With the exception of the 74.0 mg/kg dose, which was slightly lower (89%) than the specified target concentration range, each of the assayed acrylamide feed samples met the indicated specifications (Table F2).

For the 3-month study, acrylamide feed for treating the animals was prepared at 1 to 3 week intervals beginning on August 11, 2004, and ending on October 22, 2004. The target concentrations were 7.4, 18.5, 37.0, 74.0, 185, and 370 mg/kg. Concentrations were deemed acceptable if they were within 10% of the target concentrations; for the 7.4 mg/kg concentration, 20% of the target concentration was considered acceptable. The 18.5 mg/kg acrylamide feed prepared on September 29, 2004, was found to contain 87% of the desired concentration; additional analyses on a second aliquot indicated a value of 91% (Table F3). The 37 mg/kg acrylamide feed prepared on September 29, 2004, was found to contain 97% of the desired concentration; additional analyses on a second aliquot gave a value of 89% (Table F3). The 74 mg/kg acrylamide feed prepared on 29 September 2004 was found to contain 121% the desired concentration; additional analyses on a second aliquot indicated a value of 92% (Table F3).

Acrylamide was not administered in the feed in the 2-year study.

2-WEEK STUDIES

F344/N Nctr rats and B6C3F1/Nctr (C57BL/6N × C3H/HeN MTV$^-$) mice were obtained from the NCTR breeding colony at three weeks of age. The animals were tail-tattooed for identification, weight-ranked, and loaded on the MultiGen Support System. In addition, mice were ear-clipped for identification. The animals were loaded to the study in 3 balanced replicates. Treatment was initiated when the animals were 4 to 5 weeks of age. On the first day of dosing, female rats weighed between 37.3 g and 103.7 g, male rats weighed between 44.4 g and 119.5 g, female mice weighed between 12.4 g and 17.1 g, and male mice weighed between 15.1 g and 20.8 g.

Groups of four F344/N rats per sex and four B6C3F1 mice per sex were dosed with 0.0, 0.14, 0.35, 0.70, 1.41, 3.52, or 7.03 mM acrylamide in the drinking water (0, 10, 25, 50, 100, 250, or 500 ppm acrylamide) or 0.0, 7.4, 18.5, 37, 74, 185, or 370 mg acrylamide per kg diet. The animals were treated for 14 days and were monitored twice daily, in the morning and afternoon. The rats were housed two of the same sex per cage and mice were housed four of the same sex per cage. Irradiated Purina 5LG6 meal (also designated NIH-31 IR) and Millipore-filtered tap water were provided *ad libitum*. Feed was subjected to routine chemical analyses. The animal rooms were maintained on a 12-hour light-dark cycle, with 10-15 air changes per hour. Environmental controls were set to maintain the temperature at 22° ± 4° C, with a relative humidity of 40% to 70%. Body weights were recorded on dose days 1, 7, and 14. Food and water consumption was measured weekly.

On the afternoon of dose day 14, the animals were delivered to the necropsy holding area. They continued to receive dosed-water or dosed-food, depending upon the particular treatment group. On dose day 15, all animals were weighed (designated as necropsy body weight) and euthanized by exposure to carbon dioxide. Under the supervision of a pathologist, a gross examination was performed on all animals. Gross examination data were recorded with the Individual Animal Necropsy Recording system. The livers and brains were dissected and weighed. Gross lesions and the following organs were processed for microscopic examination: brain (cerebrum, cerebellum, and brain stem), harderian glands, heart, liver, lungs, pancreas, peripheral nerve (sciatic), ovaries, thyroid gland, parathyroid gland, skin, mammary glands, spinal cord (thoracic, lumbar, and cervical), forestomach, glandular stomach, and testes. The pathology data were recorded in Micropath.

3-MONTH STUDIES

F344/N Nctr rats and B6C3F1/Nctr (C57BL/6N × C3H/HeN MTV$^-$) mice were obtained from the NCTR breeding colony at three weeks of age. Rats were tail-tattooed and mice were ear-clipped for identification. Mice were also tail-tattooed at 8 to 12 weeks of age. The animals were loaded to the study in 3 balanced replicates. The animals were weight-ranked, and loaded

on the MultiGen Support System. Treatment was initiated when the rats were 4 to 5 weeks of age and the mice were 5 to 6 weeks of age. On the first day of dosing, female rats weighed between 78.8 g and 132.9 g, male rats weighed between 80.3 g and 157.9 g, female mice weighed between 13.4 g and 17.9 g and male mice weighed between 15.4 g and 24.0 g.

The rats were housed two of the same sex per cage and mice were housed four of the same sex per cage in polycarbonate cages with hardwood chip bedding. Irradiated Purina 5L6G meal and Millipore-filtered tap water were provided *ad libitum*. Feed and water were subjected to routine microbiological and chemical analyses. The animal rooms were maintained on a 12-hour light-dark cycle, with 10 to 15 air changes per hour. Environmental controls were set to maintain the temperature at 22° ± 4° C, with a relative humidity of 40% to 70%.

Each dose group consisted of eight animals per sex. In rats, the dosage groups were 0.0, 0.14, 0.35, 0.70, 1.41, or 3.52 mM acrylamide in the drinking water (0, 10, 25, 50, 100, or 250 ppm acrylamide) or 0.0, 7.4, 18.5, 37, 74, or 185 mg acrylamide per kg diet. In mice, the dosage groups were 0.0, 0.14, 0.35, 0.70, 1.41, or 3.52 mM acrylamide in the drinking water (0, 10, 25, 50, 100, or 250 ppm acrylamide) or 0.0, 18.5, 37, 74, 185, or 370 mg acrylamide per kg diet. The animals were treated for 13 weeks and were monitored twice daily, in the morning and afternoon. Body weights, food consumption, and water consumption were measured weekly.

On the afternoon before the scheduled terminal sacrifice, the animals were delivered to the necropsy holding area. They continued to receive dosed water or dosed food depending upon the particular treatment group. On the following day, all animals were weighed (designated as necropsy body weight), and euthanized by exposure to carbon dioxide. Under the supervision of a pathologist, a gross examination was performed on all animals. Gross examination data were recorded with the Individual Animal Necropsy Recording system. The livers and brains were dissected and weighed. Gross lesions and the following organs were processed for microscopic examination: brain (cerebrum, cerebellum, and brain stem), harderian glands, heart, liver, lungs, pancreas, peripheral nerve (sciatic), ovaries, thyroid gland, parathyroid gland, skin, mammary glands, spinal cord (thoracic, lumbar, and cervical), forestomach, glandular stomach, and testes. The pathology data were recorded in Micropath.

2-YEAR STUDIES

Study Design

Each dose group consisted of 48 animals per sex per species. The dosage groups were 0, 0.0875, 0.175, 0.35, and 0.70 mM acrylamide in the drinking water (0, 6.25, 12.5, 25, and 50 ppm acrylamide). The animals were treated for 2 years and were monitored twice daily, in the morning and afternoon. Body weights, food consumption, and water consumption were measured weekly.

Source and Specification of Animals

Male and female F344/N Nctr rats were obtained from the NCTR breeding colony at three weeks of age, tail-tattooed for identification, weight-ranked, and loaded on the MultiGen Support System. The animals were loaded to the study in 12 balanced replicates. Treatment was initiated when the rats were 4 to 5 weeks of age. On the first day of dosing, the female rats weighed between 52.6 g and 106.5 g; the male rats weighed between 60.1 g and 117.2 g.

Male and female B6C3F1/Nctr (C57BL/6N × C3H/HeN MTV$^-$) mice were obtained from the NCTR breeding colony at 3 weeks of age, ear-clipped for identification (at 8 to 12 weeks of age, their tails were also tattooed to provide additional identification), weight ranked, and loaded on the MultiGen Support System. The animals were loaded to the study in 12 balanced replicates. Treatment was initiated when the mice were 5 to 6 weeks of age. On the first day of dosing, the female mice weighed between 11.1 g and 17.8 g; the male mice weighed between 13.5 g and 21.2 g.

Animal Maintenance

All animal experimental procedures were performed in accordance with an animal study protocol approved by the Institutional Animal Care and Use Committee at the NCTR.

The rats were housed two of the same sex per cage in polycarbonate cages with hardwood chip bedding. The mice were housed four of the same sex per cage in polycarbonate cages with hardwood chip bedding and micro-isolator tops. Microbiological surveillance of sentinel rats and mice was conducted on a routine basis (Appendix I).

Irradiated Purina 5LG6 meal and Millipore-filtered tap water were provided *ad libitum*. Feed was subjected to

routine chemical analyses; water underwent routine microbiological surveillance.

The animal rooms were maintained on a 12-hour light-dark cycle, with 10 to 15 air changes per hour. Environmental controls were set to maintain the temperature at 22° ± 4° C, with a relative humidity of 40% to 70%. Microbiological surveillance of the animal rooms was conducted on a routine basis.

Clinical Examinations and Pathology

On the afternoon before the scheduled terminal sacrifice, the animals were delivered to the necropsy holding area. They continued to receive the dosed water. On the following day, all animals were weighed (designated as necropsy body weight) and then euthanized by exposure to carbon dioxide. Under the supervision of a pathologist, complete necropsies were performed on all terminal sacrifice animals. Complete necropsies were also performed on all animals that died naturally or that were submitted moribund prior to the scheduled terminal sacrifice. The protocol-designated tissues (see below) were examined grossly, removed, and preserved in 10% neutral buffered formalin, except the eyes and testes, which were placed in Davidson's fixative. Gross findings were recorded in the automated Gross Pathology System. The protocol-designated tissues were trimmed, processed, and embedded in Formula R® infiltrating medium, sectioned at approximately 5 microns, and stained with hematoxylin and eosin for microscopic evaluation. In a few cases, special staining procedures were applied to selected lesions to aid in characterizing the pathology changes. The protocol-designated tissues were: brain (cerebrum, cerebellum, and brain stem), harderian glands, heart, liver, lungs, pancreas, peripheral nerve (sciatic), ovaries, thyroid gland, parathyroid gland, skin, mammary glands, spinal cord (thoracic, lumbar, and cervical), forestomach, glandular stomach, duodenum, ileum, jejunum, cecum, colon, testes, kidneys, urinary bladder, spleen, prostate, trachea, esophagus, uterus, eye, aorta, nose, pituitary, preputial/clitoral glands, epididymis, lymph nodes (mesenteric and mandibular), seminal vesicles, thymus, salivary glands, bone (femur), and adrenal glands.

Upon completion of the microscopic evaluations, the pathology data were entered into the TDMSE Data Collection System. The slides, paraffin blocks, and residual wet tissues were sent to the Block and Slide Laboratory 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 group. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. A quality assessment pathologist evaluated slides of all proliferative lesions. Some differences of opinion were reconciled between the study pathologist and the quality assessment pathologist. The remaining were reviewed by a pathology working group coordinator 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).

As noted above, acrylamide is neurotoxic in experimental animals. Since no treatment-related lesions were apparent in the CNS during the original histopathologic evaluation or PWG assessment, an additional pathology quality assessment review was conducted on the original sections of brain, spinal cord, and peripheral (sciatic) nerves. This additional review was conducted by pathologists from an independent laboratory, who had special expertise in neuropathology. During this review, all changes in the nervous system were documented, regardless of their severity. Based upon these very stringent criteria, additional lesions were detected. In light of the findings of the special neuropathology quality assessment, a special PWG, consisting of 12 experienced pathologists, was convened to evaluate the results. The special PWG utilized criteria similar to those used in the special neuropathology quality assessment and concurred that all of the lesions, regardless of their severity, be added to the pathology results for the study. This recommendation was adopted.

TABLE 1
Experimental Design and Materials and Methods in the Drinking Water and Feed Studies of Acrylamide

2-Week Studies	3-Month Studies	2-Year Studies
Study Laboratory		
U.S. FDA National Center for Toxicological Research (NCTR, Jefferson, AR)	U.S. FDA National Center for Toxicological Research (NCTR, Jefferson, AR)	U.S. FDA National Center for Toxicological Research (NCTR, Jefferson, AR)
Strain and Species		
Rats: F344/N Nctr Mice: B6C3F1/Nctr (C57BL/6N × C3H/HeN MTV$^-$)	Rats: F344/N Nctr Mice: B6C3F1/Nctr (C57BL/6N × C3H/HeN MTV$^-$)	Rats: F344/N Nctr Mice: B6C3F1/Nctr (C57BL/6N × C3H/HeN MTV$^-$)
Animal Source		
NCTR breeding colony	NCTR breeding colony	NCTR breeding colony
Time Held Before Studies		
1 to 2 weeks	1 to 2 weeks	2 to 3 weeks
Average Age When Studies Began		
4 to 5 weeks	4 to 5 weeks	5 to 6 weeks
Date of First Exposure		
Rats: April 5, 12, and 19, 2004	Rats: July 21/22, 2004; August 4/5 and 18/19, 2004	Rats: May 30, 2005; June 6, 13, 20, and 27, 2005; July 7, 11, 18, and 25, 2005; and August 1, 8, and 15, 2005
Mice: April 6, 13, and 20, 2004	Mice: July 19/20, 2004; August 2/3 and 16/17, 2004	Mice: June 2, 9, 16, 23, and 30, 2005; July 7, 17, 21, and 28, 2005; and August 4, 11, and 18, 2005
Duration of Exposure		
2 weeks	13 weeks	104 weeks
Date of Last Exposure		
Rats: April 19 and 26, 2004; May 3, 2004	Rats: October 19/20, 2005; November 3/4 and 17/18, 2004	Rats: June 5, 12, 19, and 26, 2007; July 4, 10, 17, 26, and 31, 2007; and August 7, 14, and 21, 2007
Mice: April 20 and 27, 2004; May 4, 2004	Mice: October 17/18, 2004; November 1/2 and 17/18, 2004	Mice: June 3, 10, 17, and 24, 2007; July 1, 8, 15, 22, and 29, 2007; and August 5, 12, and 19, 2007
Necropsy Dates		
Rats: April 20 and 27, 2004; May 4, 2004	Rats: October 21/22, 2004; November 4/5 and 18/19, 2004	Rats: June 6, 13, 20, and 27, 2007; July 5, 11, 18, and 25, 2007; and August 1, 8, 15, and 22, 2007
Mice: April 21 and 28, 2004; May 5, 2004	Mice: October 19/20, 2004; November 2/3 and 16/17, 2004	Mice: June 4, 11, 18, and 25, 2007; July 2, 9, 16, 23, and 30, 2007; and August 6, 13, and 20, 2007
Average Age at Necropsy		
6 – 7 weeks	17 – 18 weeks	2 years
Size of Study Groups		
4 males and 4 females	8 males and 8 females	48 males and 48 females
Method of Distribution		
Animals were distributed randomly into groups of approximately equal initial body weights.	Same as 2-week studies.	Same as 2-week studies.
Animals per Cage		
Rats: 2 same sex Mice: 4 same sex	Rats: 2 same sex Mice: 4 same sex	Rats: 2 same sex Mice: 4 same sex

TABLE 1
Experimental Design and Materials and Methods in the Drinking Water and Feed Studies of Acrylamide (continued)

2-Week Studies	3-Month Studies	2-Year Studies
Method of Animal Identification		
Rats: Tail tattoo Mice: Ear clip and tail tattoo	Rats: Tail tattoo Mice: Ear clip and tail tattoo	Rats: Tail tattoo Mice: Ear clip and tail tattoo
Diet		
Irradiated Purina 5LG6 meal feed (also designated NIH-31 IR), available *ad libitum*	Same as 2-week studies	Same as 2-week studies
Water		
Millipore-filtered tap water, available *ad libitum*	Same as 2-week studies	Same as 2-week studies
Cages		
Polycarbonate cages (Lab Products, Inc., Seaford, DE and Allentown Caging and Equipment, Allentown, NJ), changed twice weekly (rats) or once weekly (mice)	Same as 2-week studies	Same as 2-week studies
Bedding		
Autoclaved hardwood chip bedding (Northeastern Products Corp., Caspian, MI), changed twice weekly (rats) or once weekly (mice)	Same as 2-week studies	Same as 2-week studies
Cage Filters		
Spunbonded polyester (Lab Products, Inc., Seaford, DE and Allentown Caging and Equipment, Allentown, NJ), changed every 2 weeks	Same as 2-week studies	Same as 2-week studies
Racks		
Stainless steel (Research Equipment Co., Bryan, TX), changed every 3 weeks	Same as 2-week studies	Same as 2-week studies
Animal Room Environment		
Temperature: $22^{o} \pm 4^{o}$C Relative humidity: 40% to 70% Room fluorescent light: 12 hours/day Room air changes: 10 to 15/hour	Temperature: $22^{o} \pm 4^{o}$C Relative humidity: 40% to 70% Room fluorescent light: 12 hours/day Room air changes: 10 to 15/hour	Temperature: $22^{o} \pm 4^{o}$C Relative humidity: 40% to 70% Room fluorescent light: 12 hours/day Room air changes: 10 to 15/hour
Exposure Concentrations		
Drinking water: 0.0, 0.14, 0.35, 0.70, 1.41, 3.52, and 7.03 mM acrylamide (0, 10, 25, 50, 100, 250, and 500 ppm acrylamide) Feed: 0.0, 7.4, 18.5, 37, 74, 185, and 370 mg acrylamide/kg diet	Drinking water: 0.0, 0.14, 0.35, 0.70, 1.41, and 3.52 mM acrylamide (0, 10, 25, 50, 100, and 250 ppm acrylamide) Feed (rats): 0.0, 7.4, 18.5, 37, 74, and 185 mg acrylamide/kg diet Feed (mice): 0.0, 18.5, 37, 74, 185, and 370 mg acrylamide/kg diet	Drinking water: 0.0, 0.0875, 0.175, 0.35, and 0.70 mM acrylamide (0, 6.25, 12.5, 25, and 50 ppm acrylamide)
Type and Frequency of Observation		
Observed twice daily; animals were weighed on dose days 1, 7, and 14; and food and water consumption measured weekly	Observed twice daily; animals were weighed weekly; and food and water consumption were measured weekly	Same as 3-month studies
Method of Sacrifice		
Carbon dioxide asphyxiation	Same as 2-week studies	Same as 2-week studies

TABLE 1
Experimental Design and Materials and Methods in the Drinking Water and Feed Studies of Acrylamide (continued)

2-Week Studies	3-Month Studies	2-Year Studies
Necropsy Necropsies were performed on all animals. Organs weighed were liver and brain. Processing for microscopic examination was performed on gross lesions, brain (cerebrum, cerebellum, and brain stem), harderian glands, heart, liver, lungs, pancreas, peripheral nerve (sciatic), ovaries, thyroid gland, parathyroid gland, skin, mammary glands, spinal cord (thoracic, lumbar, and cervical), forestomach, glandular stomach, and testes.	Necropsies were performed on all animals. Organs weighed were liver and brain. Processing for microscopic examination was performed on gross lesions, brain (cerebrum, cerebellum, and brain stem), harderian glands, heart, liver, lungs, pancreas, peripheral nerve (sciatic), ovaries, thyroid gland, parathyroid gland, skin, mammary glands, spinal cord (thoracic, lumbar, and cervical), forestomach, glandular stomach, and testes.	Necropsies were performed on all animals. Processing for microscopic examination was performed on gross lesions, brain (cerebrum, cerebellum, and brain stem), harderian glands, heart, liver, lungs, pancreas, peripheral nerve (sciatic), ovaries, thyroid gland, parathyroid gland, skin, mammary glands, spinal cord (thoracic, lumbar, and cervical), forestomach, glandular stomach, duodenum, ileum, jejunum, cecum, colon, testes, kidneys, urinary bladder, spleen, prostate, trachea, esophagus, uterus, eye, aorta, nose, pituitary, preputial/clitoral gland, epididymis, lymph nodes (mesenteric and mandibular), seminal vesicles, thymus, salivary glands, bone (femur), and adrenal glands.

Statistical Methods

Survival Analyses

The SAS Proc Lifetest procedure was used to obtain Kaplan-Meier (Kaplan and Meier, 1958) estimates of mean and median survival times and obtain plots of survival data. SAS Proc Phreg was used to conduct Cox proportional hazards regression analyses (Cox, 1972) to compare the hazard function of each dose group to that of the control group and to test for a linear trend between the hazard and acrylamide dose. The hazard for each dose group is a function of both acrylamide dose and time on study, measured in weeks.

Body Weight Analyses

The effect of acrylamide dose on body weight was investigated with the SAS Proc Mixed procedure, using a sex-stratified, repeated measures, mixed models analysis of variance (ANOVA), with dose and week main effects and a dose × week interaction effect. Within-group correlations were modeled using a heterogeneous first order autoregressive (ARH(1)) covariance structure that allows for (1) differences in the variability of animal weights over time and (2) body weights being correlated at adjacent time points to a greater extent than at distant time points. Least squares estimates of mean body weight were obtained for each dose group from weeks 4 to 104 in 4 week intervals. Standard error estimates of the least squares mean were computed using mixed models ANOVA based upon the fixed effects of dose and time, the random effects of animals within each dose (inter-animal variability), and use of the 1st order autoregressive covariance matrix. Pairwise comparisons of dose group body weight means to control group (0.0 mM acrylamide) body weight means were performed to determine if there was a difference between the control and the respective dose group means. Dunnett's adjustment (Dunnett, 1955) was used to correct for multiple pairwise comparisons to controls. Trend tests were conducted to determine if body weight means decreased or increased with increasing dose.

Water and Feed Consumption Analyses

The effect of acrylamide dose on food and water consumption was determined on a cage basis. For each cage and for each consumption period, food and water consumption were calculated by subtracting the container weight at the end of the period from the container weight at the beginning of the period. Consumption periods were grouped into 4 week study periods based on the observation date. The sum of the food and water consumption within the study period was then divided by the number of animal-days to obtain the mean food and water consumption per day for each study period for each animal. The SAS Proc Mixed procedure was used to conduct a sex-stratified, repeated measures, mixed models ANOVA, with dose and study period main effects and a dose by study

period interaction effect. Within-group correlations were modeled using a heterogeneous first order autoregressive (ARH(1)) covariance structure that allows for (1) differences over time in the variability of the amount of food and water consumed, and (2) the amount of consumed food and water being correlated to a greater extent at adjacent time points than at distant time points. Least squares estimates of the mean amount of food and water consumed were obtained for each dose group from weeks 1 to 104 in 4 week intervals. Standard error estimates of the least squares mean were computed using mixed models ANOVA based upon the fixed effects of dose and time, the random effects of animals within each dose (inter-animal variability), and use of the 1^{st} order autoregressive covariance matrix. Pairwise comparisons of the amount of food and water consumed by the dose group to that of the control group were performed to determine if there was a difference between the control and the respective dose group means. Dunnett's adjustment (Dunnett, 1955) was used to correct for multiple pairwise comparisons to controls. Trend tests were conducted to determine if the mean amount of food and water consumed decreased or increased with increasing dose.

Water consumption and body weight data were used to determine acrylamide exposure. The amount of body weight days for a cage in a consumption period was computed by first multiplying, for each animal in a cage, the body weight of the animal by the number of days the animal was on study during the same consumption period, and then summing these products over all the animals in the cage. The amount of acrylamide consumed per kg body weight per day was then calculated by dividing the amount of water consumed per cage by the number of body weight days per cage, and then converting this quantity to mg using the dose concentration and the molecular weight of acrylamide. Consumption periods were grouped into 4 week study periods based on the observation dates.

Pathology Data Analyses

Continuity-corrected Poly-3 tests (Bailer and Portier, 1988), as modified by Bieler and Williams (1993), were used to assess the age-adjusted prevalence of neoplasms. P-values for Poly-3 trend tests were one-sided. Poly-3 tests were also used to analyze the age-adjusted prevalence of nonneoplastic lesions.

RESULTS

RATS

2-WEEK STUDY

One male rat administered 7.03 mM acrylamide in the drinking water died soon after being removed from the animal room for the scheduled terminal sacrifice. Hind-leg paralysis was observed on day 14 in all rats given 7.03 mM acrylamide in the drinking water or fed 370 mg acrylamide per kg diet (Table 2). Paralysis was not observed in any other treatment groups. There were no other significant in-life observations in any of the other treatment groups.

All rats administered 7.03 mM and female rats administered 3.52 mM acrylamide in the drinking water for 14 days had significantly decreased body weights as compared to controls (Table 3). Male and female rats fed 370 mg acrylamide per kg diet for 14 days had decreased body weights (74% and 83% of controls, respectively) as compared to controls (Table 3). Water consumption generally paralleled body weight changes, with groups given the highest dose of acrylamide typically having the lowest consumption of drinking water (Table 3). The same trend occurred with feed consumption, with the exception of female rats administered acrylamide in the diet (Table 3).

Male rats administered 0.14, 0.35, 0.70, 1.41, 3.52, and 7.03 mM acrylamide in the drinking water (10, 25, 50, 100, 250, and 500 ppm acrylamide) consumed approximately 1.4, 3.8, 7.8, 15.4, 37.4 and 67.6 mg acrylamide per kg body weight per day, respectively; the comparable values for female rats were 1.7, 4.3, 8.3, 16.9, 39.4, and 70.0 mg acrylamide per kg body weight per day. Male rats fed 7.4, 18.5, 37, 74, 185, and 370 mg acrylamide per kg diet consumed approximately 1.1, 2.7, 5.3, 11.4, 22.4, and 51.7 mg acrylamide per kg body weight per day, respectively; the comparable values for female rats were 1.2, 2.7, 6.4, 11.5, 29.4, and 63.4 mg acrylamide per kg body weight per day.

Necropsy body weights, liver weights, and liver to brain weight ratios were decreased in all rats administered 7.03 mM acrylamide in the drinking water for 14 days (Table E1). The liver weights were decreased in female rats administered 3.52 mM acrylamide and the brain weights were decreased in female rats given 7.03 mM acrylamide. Necropsy body weights and liver weights were decreased in male rats fed 370 mg acrylamide per kg diet (Table E2).

There were no neoplastic findings in any of the animals. Dilatation of the urinary bladder was observed grossly in one of four male rats given 3.52 mM acrylamide in the drinking water, in three of four males and four of four females given 7.03 mM acrylamide in the drinking water, and in all rats fed 370 mg acrylamide per kg diet (Table 2). When dilatation was observed grossly, the lesion was examined microscopically, confirming the presence of the lesion with a mild to moderate average severity.

Most of the rats having dilatation of the urinary bladder had displayed hind-leg paralysis. This correlation suggested that the dilatation of the urinary bladders in these rats may have been due to impairment of neurological function rather than to a direct toxic effect on the urinary bladder; however, microscopic examination of three levels of brain, three levels of spinal cord, and sciatic nerves of all of these animals failed to reveal any morphologic changes in nervous tissue that could be attributed to acrylamide administration.

A mild to moderate average severity for degeneration of the germinal epithelium in the seminiferous tubules of the testes was noted microscopically in all male rats given 7.03 mM acrylamide in the drinking water and in two of four male rats fed 370 mg acrylamide per kg diet (Table 2). The lesion was characterized by decreased numbers of germinal cells and the presence of multinucleated spermatids in the lumens of seminiferous tubules.

TABLE 2
Incidence of Observations and Nonneoplastic Lesions in Rats in the 2-Week Acrylamide Studies[a,b]

	Drinking Water		Feed
	3.52 mM	7.03 mM	370 mg/kg
Males			
Animals initially in study	4	4	4
Hind-leg			
Paralysis	0/4	4/4	4/4
Urinary bladder			
Dilatation	1/4 (2.0)	3/4 (3.0)	4/4 (2.7)
Testes			
Seminiferous tubule degeneration	0/4	4/4 (2.7)	2/4 (2.0)
Females			
Animals initially in study	4	4	4
Hind-leg			
Paralysis	-[c]	4/4	4/4
Urinary bladder			
Dilatation	-	4/4 (2.7)	4/4 (3.0)

[a] Data are reported as the number of lesions per number of rats examined microscopically. The average severity is given in parentheses. Severity was scored as: 1 = minimal; 2 = mild; 3 = moderate; and 4 = marked.
[b] Control animals had no incidence of hind-leg paralysis. The genital and urinary systems were not examined in the controls.
[c] Animals were not examined.

TABLE 3
Survival, Body Weights, Feed Consumption, and Water Consumption of Rats in the 2-Week Drinking Water and Feed Study of Acrylamide

Treatment	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Mean Feed Consumption[c]		Mean Water Consumption[c]	
		Day 1	Day 7	Day 14		Week 1	Week 2	Week 1	Week 2
Drinking Water									
Male									
0.0 mM	4/4	96.8 ± 3.5	125.4 ± 4.3	154.7 ± 6.7		15.3 (100)	15.6 (100)	20.0 (100)	20.2 (100)
0.14 mM	4/4	99.6 ± 10.7	140.2 ± 1.4	166.0 ± 9.4	107	15.4 (101)	16.5[d] (106)	21.8 (109)	20.9 (103)
0.35 mM	4/4	96.9 ± 11.2	122.5 ± 11.7	156.8 ± 10.1	101	12.9 (84)	14.8 (95)	20.6 (103)	22.1 (109)
0.70 mM	4/4	95.1 ± 10.8	125.0 ± 9.2	157.5 ± 10.4	102	14.7 (96)	15.6 (100)	21.9 (110)	21.7 (107)
1.41 mM	4/4	97.4 ± 7.6	120.0 ± 6.3	148.1 ± 7.9	96	13.8 (90)	16.2 (104)	21.0 (105)	19.6 (97)
3.52 mM	4/4	100.7 ± 7.4	122.8 ± 7.7	142.0 ± 7.6	92	13.7 (90)	15.1 (97)	20.5 (103)	18.7 (93)
7.03 mM	3/4[e]	96.0 ± 6.2	94.5 ± 8.5	86.6 ± 11.4*	56	10.3 (67)	7.1 (46)	15.1 (76)	9.6 (48)
Female									
0.0 mM	4/4	92.2 ± 1.8	111.5 ± 1.5	128.8 ± 2.2		13.1 (100)	12.6 (100)	20.4 (100)	18.0 (100)
0.14 mM	4/4	88.1 ± 8.4	109.5 ± 5.2	127.1 ± 4.7	99	13.2 (101)	11.7[d] (93)	22.0 (108)	18.6 (103)
0.35 mM	4/4	88.4 ± 7.7	108.1 ± 4.2	124.7 ± 3.2	97	11.9 (91)	11.6 (92)	20.3 (100)	19.5 (108)
0.70 mM	4/4	87.4 ± 7.7	103.0 ± 7.2	119.9 ± 8.0	93	12.0 (92)	11.3 (90)	18.3 (90)	18.7 (104)
1.41 mM	4/4	89.4 ± 4.5	104.1 ± 2.9	118.4 ± 2.3	92	11.4 (87)	11.7 (93)	19.7 (97)	17.5 (97)
3.52 mM	4/4	90.1 ± 4.7	98.2 ± 4.1	109.4 ± 4.2*	85	10.7 (82)	11.8 (94)	17.4 (85)	15.1 (84)
7.03 mM	4/4	93.2 ± 1.8	88.9 ± 2.1*	82.1 ± 3.4*	64	7.9 (60)	7.5 (60)	15.6 (76)	8.6 (48)
Feed									
Male									
0 mg/kg	4/4	64.4 ± 6.4	102.1 ± 7.4	135.9 ± 7.0		14.6 (100)	18.3 (100)	18.4 (100)	19.5 (100)
7.4 mg/kg	4/4	65.7 ± 11.3	101.7 ± 13.8	131.4 ± 14.5	97	16.8 (115)	17.8 (97)	17.6 (96)	18.3 (94)
18.5 mg/kg	4/4	63.6 ± 10.0	100.3 ± 11.5	130.9 ± 11.7	96	15.2 (104)	18.6 (102)	16.4 (89)	21.6 (111)
37 mg/kg	4/4	66.9 ± 6.4	102.8 ± 6.1	135.1 ± 5.8	99	15.2 (104)	18.8 (103)	19.1 (104)	20.1 (103)
74 mg/kg	4/4	64.7 ± 2.6	97.0 ± 3.2	125.7 ± 4.4	92	15.8 (108)	18.1 (99)	16.6 (90)	18.7 (96)
185 mg/kg	4/4	64.8 ± 2.7	95.6 ± 4.7	121.5 ± 6.0	89	14.5 (99)	18.6 (102)	17.2 (93)	20.6 (106)
370 mg/kg	4/4	64.4 ± 1.8	87.0 ± 3.2	100.5 ± 3.7	74	11.4 (78)	14.9 (81)	15.4 (84)	14.6 (75)

TABLE 3
Survival, Body Weights, Feed Consumption, and Water Consumption of Rats in the 2-Week Drinking Water and Feed Study of Acrylamide (continued)

Treatment	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Mean Feed Consumption[c]		Mean Water Consumption[c]	
		Day 1	Day 7	Day 14		Week 1	Week 2	Week 1	Week 2
Feed (continued)									
Female									
0 mg/kg	4/4	56.6 ± 2.8	84.8 ± 3.7	105.2 ± 4.6		14.3 (100)	15.6 (100)	15.4 (100)	18.3 (100)
7.4 mg/kg	4/4	60.7 ± 8.7	88.0 ± 8.3	109.3 ± 7.5	104	14.9 (104)	15.8 (101)	15.5 (101)	18.7 (102)
18.5 mg/kg	4/4	60.4 ± 7.6	88.3 ± 7.1	107.6 ± 5.9	102	13.1 (92)	15.6 (100)	15.8 (103)	17.9 (98)
37 mg/kg	4/4	50.7 ± 6.3	83.9 ± 4.8	106.8 ± 4.0	102	16.0 (112)	16.8 (108)	15.1 (98)	18.8 (103)
74 mg/kg	4/4	59.7 ± 2.3	88.2 ± 4.1	107.0 ± 6.0	102	14.0 (98)	16.4 (105)	15.4 (100)	17.7 (97)
185 mg/kg	4/4	60.8 ± 1.6	87.7 ± 2.9	106.2 ± 3.7	101	14.0 (98)	16.8 (108)	16.7 (108)	18.8 (103)
370 mg/kg	4/4	58.8 ± 2.2	78.1 ± 2.1	87.8 ± 2.8	83	12.6 (88)	15.9 (102)	13.5 (88)	12.2 (67)

[a] Number of animals surviving at 14 days/number initially in group.
[b] Weights are given as mean ± standard error. An asterisk (*) denotes those that are significantly different ($p < 0.05$) from controls.
[c] Feed and water consumption are expressed as grams per animal per day and were measured on a per cage basis and presented as mean of two cages and, in parentheses, the percentage of the respective control. Statistical analyses were not conducted on feed and water consumption because there were only two cages per treatment group.
[d] Data based upon one cage only.
[e] One animal died in pathology prior to sacrifice.

Exposure Concentration Selection Rationale: Based upon incidence of hind-leg paralysis and decreased body weight at 7.03 mM acrylamide in drinking water, a high dose of 3.52 mM acrylamide (250 ppm acrylamide) was selected for the 3-month subchronic drinking water study, with the remaining doses being 0, 0.14, 0.35, 0.70, and 1.41 mM acrylamide (0, 10, 25, 50, and 100 ppm acrylamide). Based upon incidence of hind-leg paralysis and decreased body weight at 370 mg acrylamide per kg diet, a high dose of 185 mg acrylamide per kg diet was selected for the 3-month subchronic feeding study, with the remaining doses being 0, 7.4, 18.5, 37, and 74 mg acrylamide per kg diet.

3-MONTH STUDY

All animals survived to the end of the 13-week experiment. Hind-leg paralysis was observed after 4 weeks of treatment in rats administered 3.52 mM acrylamide, after 10 weeks in two of eight female rats administered 1.41 mM acrylamide, and after 13 weeks in four of eight females administered 1.41 mM acrylamide in the drinking water. In rats administered 185 mg acrylamide per kg diet, hind-leg paralysis was observed after approximately 7 weeks of treatment.

Acrylamide in the drinking water or diet caused significant dose-related effects on body weight in rats (Table 4 and Figures 5 and 6). Treatment with 3.52 mM acrylamide and 185 mg acrylamide per kg diet resulted in significant decreases in body weight gain in male and female rats and with 1.41 mM acrylamide resulted in significant decreases in body weight gain in female rats. Mean body weights in the group treated with 3.52 mM acrylamide in drinking water were depressed by more than 10% after 4 (males) to 5 (females) weeks of dosing and at the end of the 13-week period, the rats weighed 71% (females) to 73% (males) of controls. Treatment with 1.41 mM acrylamide resulted in significant decreases in body weight gain in female rats. Mean body weights in the 185 mg acrylamide per kg diet group were depressed by more than 10% after 3 (males) to 7 (females) weeks of dosing and at the end of the 13-week period, the rats weighed 82% (females) to 86% (males) of controls (Figure 6).

Necropsy body weights and brain weights were decreased and liver weight to body weight ratios were increased in rats administered 3.52 mM acrylamide in the drinking water for 13 weeks (Table E3). Liver weights and liver weight to brain weight ratios were decreased in male rats administered 3.52 mM acrylamide and necropsy body weights were decreased in female rats administered 1.41 mM acrylamide. Necropsy body weights were decreased in rats fed 185 mg acrylamide per kg diet for 13 weeks (Table E4). In the 185 mg acrylamide per kg diet group, liver and brain weights were decreased in female rats and the liver weight to body weight ratios were increased in male rats.

Acrylamide in the drinking water caused significant dose effects on water consumption in rats (Table 5). Treatment with 3.52 mM acrylamide resulted in significant decreases in water consumption compared to the control group, with the decrease becoming evident after 6 weeks in male rats and 5 weeks in female rats. Acrylamide in the diet caused significant dose effects on water consumption in rats (Table 6); however, there were no significant differences when pairwise comparisons were conducted.

Acrylamide in the drinking water caused significant dose effects on food consumption in male and female rats (Table 7). Treatment with 3.52 mM acrylamide resulted in significant decreases in food consumption compared to the respective control group, with the decrease being evident at all time points measured. Acrylamide in the diet caused significant dose effects on food consumption in male but not female rats (Table 8). Treatment with 185 mg acrylamide per kg diet resulted in significant decrease in food consumption in male rats.

Male rats administered 0.14, 0.35, 0.70, 1.41, and 3.52 mM acrylamide in the drinking (10, 25, 50, 100, or 250 ppm acrylamide) consumed approximately 0.8, 2.1, 4.5, 8.6, and 22.3 mg acrylamide per kg body weight per day; the comparable values for female rats were 1.1, 2.7, 6.0, 12.3, and 26.3 mg acrylamide per kg body weight per day. Male rats fed 7.4, 18.5, 37, 74, and 185 mg acrylamide per kg diet consumed approximately 0.5, 1.4, 2.8, 5.5, and 14.2 mg acrylamide per kg body weight per day; the comparable values for female rats were 0.6, 1.6, 3.2, 6.6, and 17.9 mg acrylamide per kg body weight per day.

There were no neoplastic findings in any of the animals. The only gross observation that was considered to be treatment-related was marked dilatation of the urinary bladder of all male rats and six of eight female rats administered 3.52 mM acrylamide. This same observation was noted in three of eight male rats and three of seven female rats fed 185 mg acrylamide per kg diet. All of these animals had a clinical observation of partial paralysis of the hind legs.

In rats administered acrylamide in the drinking water, treatment-related changes were observed in the following target tissues: sciatic nerve, spinal cord, skeletal muscle of the hind-limb, spleen, bone marrow, testes, epididymis, ovary, and uterus. In rats administered acrylamide in the diet, treatment-related changes were observed in the sciatic nerve, spinal cord, skeletal muscle of the hind-limb, testes, and epididymis. Target tissues were examined microscopically in progressively lower dose groups until a no-observed-effect level was reached. The most significant treatment-related change was radiculoneuropathy (a degenerative lesion) involving the sciatic nerve and lumbar spinal cord in all rats administered 3.52 mM acrylamide in drinking water and involving the sciatic

TABLE 4
Survival and Body Weights of Rats in the 3-Month Drinking Water and Feed Studies of Acrylamide

Treatment	Survival[a]	Mean Body Weight[b] (g)		Final weight Relative to Controls (%)
		Week 0	Week 14	
Drinking Water				
Male				
0.0 mM	8/8	130.0 ± 2.5	333.2 ± 2.5	
0.14 mM	8/8	135.0 ± 2.5	345.1 ± 2.9	104
0.35mM	8/8	133.7 ± 2.5	335.2 ± 2.5	101
0.70 mM	8/8	132.7 ± 2.5	335.5 ± 2.5	101
1.41 mM	8/8	133.8 ± 2.5	320.4 ± 2.5	96
3.52 mM	8/8	135.6 ± 2.5	241.4 ± 2.5	73
Female				
0.0 mM	8/8	107.5 ± 1.6	199.9 ± 1.6	
0.14 mM	8/8	112.5 ± 1.6	204.2 ± 1.6	102
0.35 mM	8/8	109.4 ± 1.6	194.4 ± 1.6	97
0.70 mM	8/8	111.0 ± 1.6	194.0 ± 1.6	97
1.41 mM	8/8	108.0 ± 1.6	182.8 ± 1.6	91
3.52 mM	8/8	109.6 ± 1.6	141.3 ± 1.6	71
Feed				
Male				
0 mg/kg	8/8	124.5 ± 2.5	345.7 ± 2.5[c]	
7.4 mg/kg	8/8	125.5 ± 2.5	350.4 ± 2.5[c]	101
18.5 mg/kg	8/8	121.9 ± 2.5	336.2 ± 2.5[c]	97
37 mg/kg	8/8	130.4 ± 2.5	347.4 ± 2.5[c]	101
74 mg/kg	8/8	129.3 ± 2.5	340.2 ± 2.5[c]	98
185 mg/kg	8/8	124.6 ± 2.5	296.4 ± 2.5[c]	86
Female				
0 mg/kg	8/8	104.7 ± 1.7	203.7 ± 1.7	
7.4 mg/kg	8/8	104.8 ± 1.7	205.0 ± 1.7	101
18.5 mg/kg	8/8	106.8 ± 1.7	198.2 ± 1.7	97
37 mg/kg	8/8	105.1 ± 1.7	198.4 ± 1.7	97
74 mg/kg	8/8	104.7 ± 1.7	192.5 ± 1.7	95
185 mg/kg	8/8	103.2 ± 1.7	166.4 ± 1.7	82

[a] Number of animals surviving until study termination/number of animals initially in group.
[b] Weights are given as LS means ± standard error of the mean.
[c] Final male body weights in the feed study are from week 13.

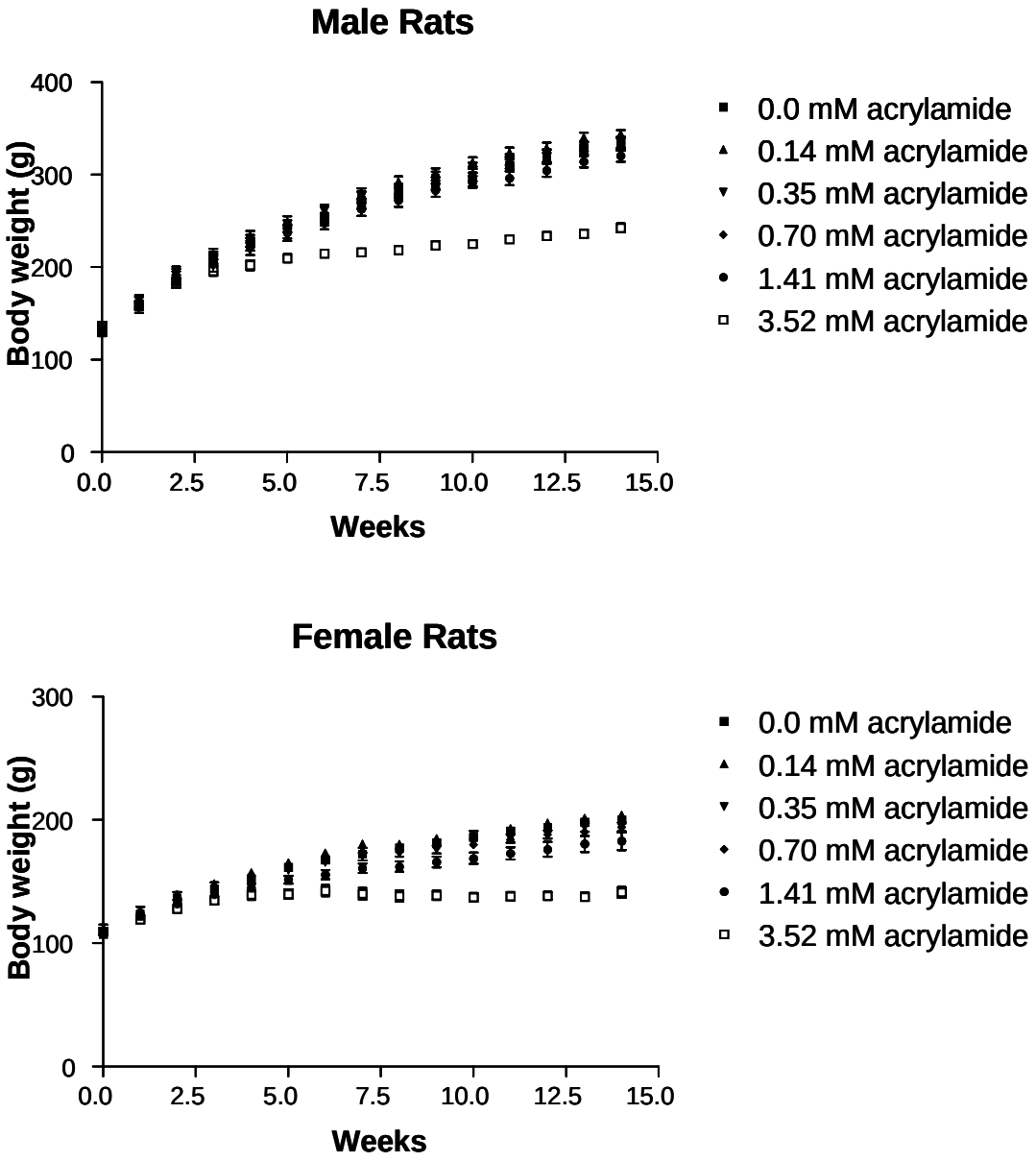


FIGURE 5
Growth Curves for Male and Female Rats
in the 3-Month Drinking Water Study of Acrylamide

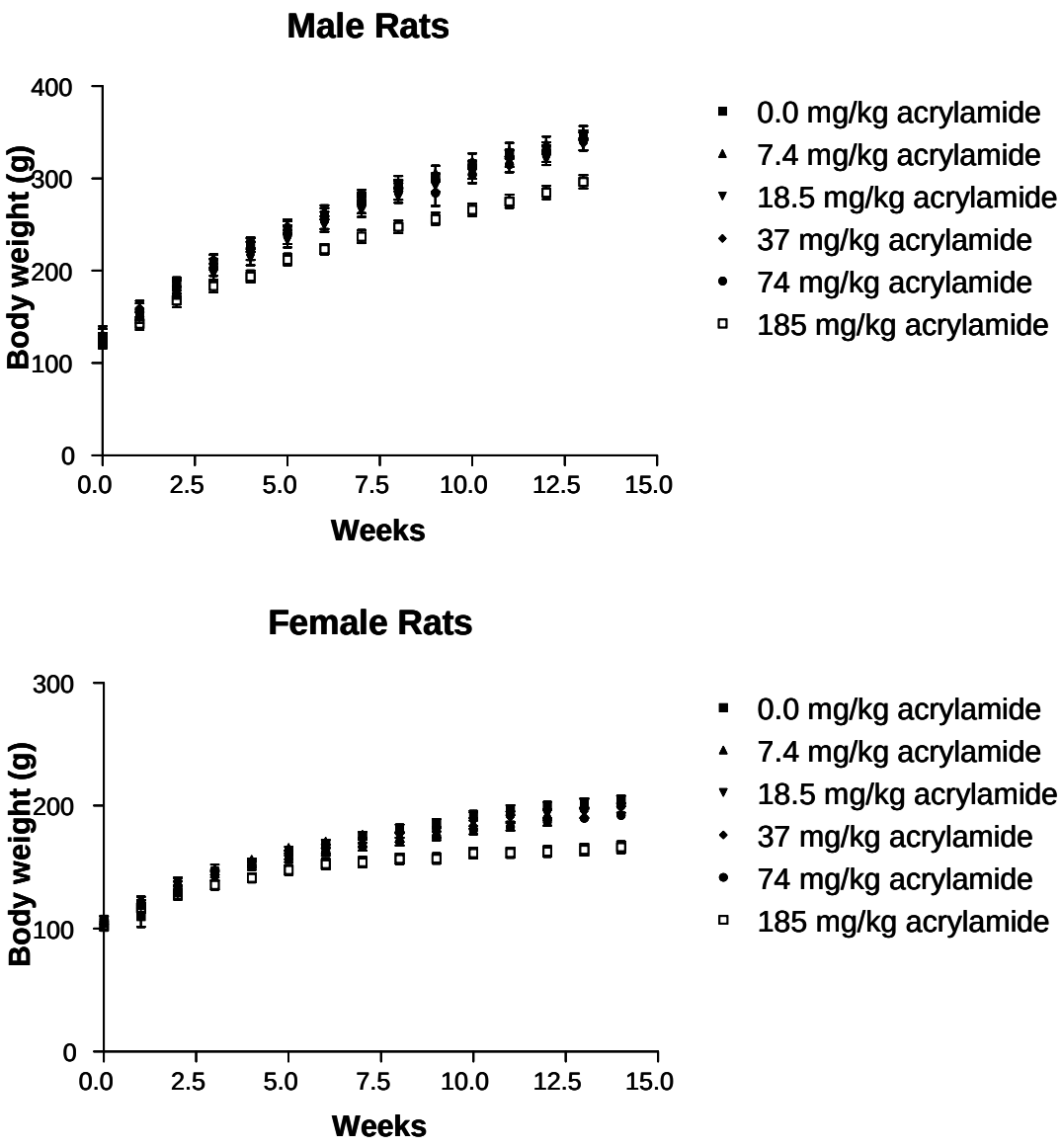


FIGURE 6
Growth Curves for Male and Female Rats in the 3 -Month Feed Study of Acrylamide

TABLE 5
Water Consumption of Rats in the 3-Month Drinking Water Study of Acrylamide[a]

Week	0 mM	0.14 mM	0.35 mM	0.70 mM	1.41 mM	3.52 mM
Male						
2	19.7 ± 0.7	22.7 ± 0.7	21.6 ± 0.7	22.8 ± 0.7	22.2 ± 0.7	21.1 ± 0.7
3	22.1 ± 0.7	22.6 ± 0.7	23.2 ± 0.7	24.2 ± 0.7	23.2 ± 0.7	24.2 ± 0.7
4	22.3 ± 0.7	22.3 ± 0.7	23.5 ± 0.7	23.7 ± 0.7	23.5 ± 0.7	24.1 ± 0.7
5	22.1 ± 0.7	21.4 ± 0.7	23.8 ± 0.7	25.3 ± 0.7	22.6 ± 0.7	23.0 ± 0.7
6	22.2 ± 0.7	22.0 ± 0.7	23.2 ± 0.7	24.5 ± 0.7	21.6 ± 0.7	21.1 ± 0.7
7	21.7 ± 0.7	23.0 ± 0.7	24.8 ± 0.7	23.4 ± 0.7	22.0 ± 0.7	18.1 ± 0.7
8	20.1 ± 0.7	21.5 ± 0.7	21.7 ± 0.7	22.8 ± 0.7	20.0 ± 0.7	16.2 ± 0.7
9	19.6 ± 0.7	21.5 ± 0.7	22.3 ± 0.7	23.4 ± 0.7	19.5 ± 0.7	14.6 ± 0.7
10	21.9 ± 0.7	21.8 ± 0.7	23.5 ± 0.7	24.2 ± 0.7	20.4 ± 0.7	16.5 ± 0.7
11	20.7 ± 0.7	21.6 ± 0.7	20.7 ± 0.7	23.1± 0.7	20.6 ± 0.7	15.9 ± 0.7
12	19.9 ± 0.7	21.1 ± 0.7	21.1 ± 0.7	22.2 ± 0.7	21.2 ± 0.7	15.2 ± 0.7
13	21.9 ± 0.7	21.4 ± 0.7	21.0 ± 0.7	22.2 ± 0.7	22.9 ± 0.7	17.4 ± 0.7
Female						
2	18.9 ± 0.7	21.0 ± 0.7	20.7 ± 0.7	20.8 ± 0.7	19.8 ± 0.7	18.8 ± 0.7
3	19.4 ± 0.7	20.7 ± 0.7	19.5 ± 0.7	21.6 ± 0.7	20.3 ± 0.7	19.6 ± 0.7
4	20.5 ± 0.7	19.5 ± 0.7	18.6 ± 0.7	20.8 ± 0.7	20.1 ± 0.7	17.7 ± 0.7
5	19.8 ± 0.7	18.3 ± 0.7	20.1 ± 0.7	20.5 ± 0.9	23.3 ± 0.7	15.9 ± 0.7
6	19.1 ± 0.7	20.0 ± 0.7	17.2 ± 0.7	20.5 ± 0.7	19.3 ± 0.7	13.8 ± 0.7
7	19.2 ± 0.7	20.0 ± 0.7	18.2 ± 0.7	19.9 ± 0.7	19.6 ± 0.7	12.9 ± 0.7
8	17.2 ± 0.7	18.1 ± 0.7	16.8 ± 0.7	19.7 ± 0.7	18.1 ± 0.7	12.5 ± 0.7
9	16.5 ± 0.7	18.2 ± 0.7	16.4 ± 0.7	20.0 ± 0.7	17.1 ± 0.7	11.9 ± 0.7
10	18.3 ± 0.7	17.5 ± 0.7	17.4 ± 0.7	20.0 ± 0.7	19.2 ± 0.7	13.1 ± 0.7
11	18.9 ± 0.7	17.5 ± 0.7	17.1 ± 0.7	19.8 ± 0.7	18.6 ± 0.7	12.5 ± 0.7
12	17.0 ± 0.7	17.0 ± 0.7	17.2 ± 0.7	19.7 ± 0.7	17.2 ± 0.7	11.6 ± 0.7
13	18.1 ± 0.7	17.3 ± 0.7	18.1 ± 0.7	19.9 ± 0.7	18.5 ± 0.7	13.3 ± 0.9

[a] Water consumption is given as LS mean ± standard error of the mean and is expressed as grams per animal per day.

TABLE 6
Water Consumption of Rats in the 3-Month Feed Study of Acrylamide[a]

Week	0 mg/kg	7.4 mg/kg	18.5 mg/kg	37 mg/kg	74 mg/kg	185 mg/kg
Male						
4	22.4 ± 0.9	21.3 ± 0.9	22.8 ± 0.9	22.2 ± 0.9	22.6 ± 0.9	21.0 ± 0.9
5	21.1 ± 0.9	19.3 ± 0.9	16.4 ± 0.9	23.1 ± 0.9	20.3 ± 0.9	17.4 ± 0.9
6	21.4 ± 0.9	21.6 ± 0.9	19.6 ± 0.9	23.4 ± 0.9	22.3 ± 0.9	19.8 ± 0.9
7	22.1 ± 0.9	21.8 ± 0.9	22.7 ± 0.9	24.8 ± 0.9	22.2 ± 0.9	19.8 ± 0.9
8	21.6 ± 0.9	23.0 ± 0.9	23.0 ± 0.9	22.8 ± 0.9	22.1 ± 0.9	20.0 ± 0.9
9	20.4 ± 0.9	23.0± 0.9	21.9 ± 0.9	23.6 ± 0.9	23.1 ± 0.9	18.4 ± 0.9
11	21.6 ± 0.9	22.2 ± 0.9	21.8 ± 0.9	24.1 ± 0.9	24.4 ± 0.9	18.2 ± 0.9
12	21.9 ± 0.9	23.4 ± 0.9	22.2 ± 0.9	23.7 ± 0.9	24.4 ± 0.9	18.8 ± 0.9
13	19.7 ± 0.9	21.4 ± 0.9	23.0 ± 0.9	22.7 ± 0.9	14.4 ± 0.9	19.3 ± 0.9
Female						
4	19.2 ± 0.8	18.6 ± 0.8	18.8 ± 0.8	18.5 ± 0.8	19.8 ± 0.8	18.2 ± 0.8
5	19.8 ± 0.8	17.5 ± 0.8	19.2 ± 0.8	17.5 ± 0.8	18.0 ± 0.8	15.8 ± 1.0
6	19.0 ± 0.8	18.4 ± 0.8	18.6 ± 0.8	20.4 ± 0.8	17.6 ± 0.8	16.5 ± 0.8
7	18.3 ± 0.8	19.5 ± 0.8	19.6 ± 0.8	18.4 ± 0.8	18.2 ± 0.8	16.3 ± 0.8
8	18.3 ± 0.8	18.0 ± 0.8	19.2 ± 0.8	18.4 ± 0.8	17.8 ± 0.8	15.8 ± 0.8
9	16.3 ± 0.8	18.8 ± 0.8	19.6 ± 0.8	18.9 ± 0.8	18.2 ± 0.8	15.9 ± 0.8
10	16.0 ± 0.8	21.2 ± 0.8	19.0 ± 0.8	17.8 ± 0.8	17.7 ± 0.8	15.5 ± 0.8
11	17.1 ± 0.8	17.6 ± 0.8	19.5 ± 0.8	18.6 ± 0.8	17.2 ± 0.8	16.5 ± 0.8
12	17.6 ± 0.8	19.1 ± 0.8	20.6 ± 0.8	18.5 ± 0.8	18.5 ± 0.8	16.4 ± 0.8
13	15.3 ± 1.0	18.8 ± 0.8	17.7 ± 0.8	16.3 ± 0.8	19.6 ± 0.8	18.2 ± 0.8

[a] Water consumption is given as LS mean ± standard error of the mean and is expressed as grams per animal per day.

TABLE 7
Feed Consumption of Rats in the 3-Month Drinking Water Study of Acrylamide[a]

Week	0 mM	0.14 mM	0.35 mM	0.70 mM	1.41 mM	3.52 mM
Male						
5	16.8 ± 0.6	17.4 ± 0.6	17.3 ± 0.6	17.8 ± 0.6	17.3 ± 0.6	14.8 ± 0.6
6	16.2 ± 0.6	16.3 ± 0.6	16.1 ± 0.6	17.6 ± 0.6	15.1 ± 0.6	14.8 ± 0.6
7	17.4 ± 0.6	16.4 ± 0.6	16.3 ± 0.6	17.3 ± 0.6	16.8 ± 0.6	14.2 ± 0.6
8	17.2 ± 0.6	17.0 ± 0.6	15.9 ± 0.6	17.6 ± 0.7	15.5 ± 0.6	13.2 ± 0.6
9	15.2 ± 0.6	14.7 ± 0.6	16.7 ± 0.6	15.5 ± 0.6	14.8 ± 0.6	13.3 ± 0.6
10	17.1 ± 0.6	16.6 ± 0.6	18.0 ± 0.6	18.5 ± 0.6	16.3 ± 0.6	14.0 ± 0.6
11	16.4 ± 0.6	16.7 ± 0.6	16.3 ± 0.6	17.5 ± 0.6	15.9 ± 0.6	13.1 ± 0.6
12	17.6 ± 0.6	17.4 ± 0.6	16.5 ± 0.6	18.0 ± 0.6	16.4 ± 0.6	13.8 ± 0.6
13	17.9 ± 0.6	16.7 ± 0.6	16.7 ± 0.6	17.4 ± 0.6	16.9 ± 0.6	14.6 ± 0.6
Female						
5	11.7 ± 0.4	12.8 ± 0.4	12.2 ± 0.4	13.6 ± 0.4	11.9 ± 0.4	10.8 ± 0.4
6	12.0 ± 0.4	12.3 ± 0.4	11.6 ± 0.4	12.6 ± 0.5	11.8 ± 0.4	9.3 ± 0.4
7	12.3 ± 0.4	12.7 ± 0.4	13.3 ± 0.4	12.3 ± 0.4	11.2 ± 0.4	9.3 ± 0.4
8	12.4 ± 0.4	13.6± 0.4	11.4 ± 0.4	12.1 ± 0.4	12.3 ± 0.4	11.0 ± 0.4
9	12.1 ± 0.4	11.6 ± 0.4	12.0 ± 0.4	13.0 ± 0.5	13.1 ± 0.4	9.9 ± 0.4
10	13.2 ± 0.4	12.2 ± 0.4	11.7 ± 0.4	12.2 ± 0.4	12.6 ± 0.4	10.5 ± 0.4
11	12.0 ± 0.4	13.3 ± 0.4	12.5 ± 0.4	12.4 ± 0.4	11.3 ± 0.4	9.9 ± 0.4
12	12.8 ± 0.4	12.6 ± 0.4	12.4 ± 0.4	13.1 ± 0.4	13.0 ± 0.4	9.9 ± 0.4
13	12.6 ± 0.4	12.6 ± 0.4	11.8 ± 0.4	13.2 ± 0.4	12.0 ± 0.4	9.8 ± 0.4

[a] Feed consumption is given as LS mean ± standard error of the mean and is expressed as grams per animal per day.

TABLE 8
Feed Consumption of Rats in the 3-Month Feed Study of Acrylamide[a]

Week	0 mg/kg	7.4 mg/kg	18.5 mg/kg	37 mg/kg	74 mg/kg	185 mg/kg
Male						
1	14.4 ± 0.6	15.1 ± 0.6	15.1 ± 0.6	15.9 ± 0.6	14.9 ± 0.6	14.7 ± 0.6
2	15.0 ± 0.6	15.7 ± 0.6	15.9 ± 0.6	16.8 ± 0.6	16.8 ± 0.6	14.4 ± 0.6
3	18.7 ± 0.6	18.7 ± 0.6	18.6 ± 0.7	19.4 ± 0.6	19.7 ± 0.6	19.4 ± 0.6
4	17.8 ± 0.6	17.7 ± 0.6	17.1 ± 0.6	19.7 ± 0.6	18.7 ± 0.6	17.5 ± 0.6
5	18.9 ± 0.6	17.1 ± 0.6	16.5 ± 0.6	19.1 ± 0.6	18.1 ± 0.6	16.1 ± 0.6
6	19.0 ± 0.6	18.4 ± 0.6	17.8 ± 0.6	19.5 ± 0.6	17.4 ± 0.6	17.2 ± 0.6
7	18.7 ± 0.6	17.9 ± 0.6	16.8 ± 0.6	18.2 ± 0.6	17.4 ± 0.6	16.4 ± 0.6
8	19.2 ± 0.6	18.9 ± 0.6	18.3 ± 0.6	19.4 ± 0.6	18.6 ± 0.6	17.5 ± 0.6
9	19.7 ± 0.6	19.8 ± 0.6	19.1 ± 0.6	20.2 ± 0.6	19.3 ± 0.6	18.2 ± 0.6
10	19.4 ± 0.6	18.8 ± 0.6	19.2 ± 0.6	19.7 ± 0.6	17.3 ± 0.6	17.3 ± 0.6
11	22.0 ± 0.6	20.2 ± 0.6	21.0 ± 0.6	22.4 ± 0.6	22.2 ± 0.6	18.0 ± 0.6
12	20.6 ± 0.6	21.2 ± 0.6	20.3 ± 0.6	21.5 ± 0.6	22.8 ± 0.6	17.0 ± 0.6
13	19.7 ± 0.6	21.3 ± 0.6	19.6 ± 0.6	21.4 ± 0.7	22.1 ± 0.6	18.6 ± 0.6
Female						
1	12.5 ± 0.5	12.3 ± 0.5	12.5 ± 0.5	13.0 ± 0.5	12.2 ± 0.5	12.9 ± 0.6
2	12.4 ± 0.6	12.9 ± 0.5	13.8 ± 0.5	13.5 ± 0.5	13.6 ± 0.5	13.5 ± 0.5
3	13.5 ± 0.5	14.8 ± 0.5	16.0 ± 0.5	15.1 ± 0.5	15.9 ± 0.5	16.9 ± 0.5
4	14.1 ± 0.5	14.1 ± 0.5	13.7 ± 0.5	13.5 ± 0.5	13.7 ± 0.5	13.6 ± 0.5
5	13.2 ± 0.5	13.7 ± 0.5	13.3 ± 0.5	13.5 ± 0.5	15.3 ± 0.5	15.0 ± 0.5
6	14.7 ± 0.5	15.1 ± 0.5	15.1 ± 0.5	14.6 ± 0.5	13.9 ± 0.5	14.7 ± 0.5
7	13.5 ± 0.5	13.8 ± 0.5	12.8 ± 0.5	13.9 ± 0.5	13.3 ± 0.5	13.8 ± 0.5
8	14.5 ± 0.5	13.5 ± 0.5	13.7 ± 0.5	13.7 ± 0.5	13.5 ± 0.5	14.0 ± 0.5
9	14.6 ± 0.5	14.0 ± 0.5	15.3 ± 0.5	14.2 ± 0.5	14.9 ± 0.5	15.0 ± 0.5
10	14.7 ± 0.5	15.0 ± 0.6	14.1 ± 0.5	13.5 ± 0.5	15.6 ± 0.5	13.9 ± 0.5
11	17.9 ± 0.5	15.6 ± 0.5	16.2± 0.5	15.0 ± 0.5	16.7 ± 0.5	15.7 ± 0.5
12	16.5 ± 0.5	15.1 ± 0.5	16.0 ± 0.5	15.9 ± 0.5	14.2 ± 0.5	13.8 ± 0.5
13	14.9 ± 0.5	15.5 ± 0.5	14.4 ± 0.5	14.6 ± 0.6	15.7 ± 0.5	13.8 ± 0.5

[a] Feed consumption is given as LS mean ± standard error of the mean and is expressed as grams per animal per day.

nerve in rats administered 185 mg acrylamide per kg diet (Tables 9 and 10). The lesion was also observed in two of eight female rats fed 74 mg acrylamide per kg diet. The radiculoneuropathy was characterized by nerve fiber degeneration with dilatation and vacuolization of myelin sheaths along with swollen and shrunken axons. The severity of these changes was minimal to moderate. The neuronal degenerative changes were accompanied by minimal to mild atrophy in skeletal muscle of the hind-limb due to decreased myofiber size in six of eight male rats and seven of eight female rats administered 3.52 mM acrylamide in the drinking water. Luminal dilation of the urinary bladder was also diagnosed in most of the same animals. Degenerative changes in the spinal cord were observed in one of eight female rats fed 185 mg acrylamide per kg diet. The neuronal degenerative changes were accompanied by minimal to mild atrophy in skeletal muscle of the hind-limb all male rats and seven of eight female rats fed 185 mg acrylamide per kg diet. Luminal dilation of the urinary bladder was also diagnosed in three of eight male rats and three of seven female rats fed 185 mg acrylamide per kg diet.

Treatment-related histopathological lesions were observed in sections of spleens and bone marrow of rats treated with acrylamide in the drinking water (Table 9). In the spleen, there was sequestration of red blood cells in sinuses and small vessels. Some of the sequestered erythrocytes may have been phagocytized by cells lining the sinuses. Most of the sequestered erythrocytes were a dull brown color rather than the bright red that characterized normal red blood cells. There was also an apparent increase in hemosiderin pigment in these spleens. This change was present in all rats treated with 3.52 mM acrylamide and in two of eight male rats fed 185 mg acrylamide per kg diet. In bone sections of rats treated with 3.52 mM acrylamide, there was minimal to mild hyperplasia of red blood cell precursors suggesting a response to anemia. The bone marrow response was limited to rats administered 3.52 mM acrylamide.

Degeneration of the germ cells in the testes was observed in all male rats given 3.52 mM and 1.41 mM acrylamide and in five of eight male rats treated with 0.70 mM acrylamide, and in all dose groups of male rats fed diet containing acrylamide, with the incidence increasing with increasing dose (Tables 9 and 10). The average severity of the degenerative change was mild to moderate in the 3.52 mM group and minimal-to-mild in the 1.41 and 0.70 mM groups and in the acrylamide diet groups. A corresponding lesion that consisted of exfoliated degenerating germ cells, cellular debris, and hypospermia was observed in the epididymides of most of these rats (Tables 9 and 10).

Examination of the female reproductive organs indicated that all of the animals administered 3.52 mM acrylamide in the drinking water were in anestrus (Table 9). In the ovary, anestrus was characterized by the lack of corpora lutea in various stages of development and regression from subsequent ovulations. The uteri of these rats were characterized by endometrium and endometrial glands lined by low cuboidal epithelium with a marked reduction in mitotic activity indicating lack of cyclic change. No animals in the dosed feed study were in anestrus.

TABLE 9
Incidence of Nonneoplastic Lesions in Rats in the 3-Month Drinking Water Study of Acrylamide[a]

	0 mM	0.14 mM	0.35 mM	0.70 mM	1.41 mM	3.52 mM
Males						
Animals initially in study	8	8	8	8	8	8
Peripheral nerve						
Axon degeneration	0/8	-[b]	-	-	0/8	8/8 (3.0)
Schwann cell degeneration	0/8	-	-	-	0/8	8/8 (3.0)
Spinal cord						
Lumbar axon degeneration	0/8	-	-	-	-	8/8 (1.9)
Skeletal muscle						
Atrophy	0/8	-	-	-	0/8	6/8 (1.8)
Urinary bladder						
Dilatation	0/8	-	-	-	-	8/8 (3.1)
Spleen						
Congestion	0/8	-	-	-	0/8	7/8 (2.7)
Pigmentation	0/8	-	-	-	0/8	8/8 (2.8)
Bone marrow						
Erythroid cell hyperplasia	0/8	-	-	-	0/8	8/8 (2.0)
Testes						
Germinal epithelium degeneration	0/8	-	0/8	5/8 (1.0)	8/8 (1.0)	8/8 (2.8)
Epididymis						
Exfoliated germ cell	0/8	-	0/8	2/8 (1.0)	8/8 (1.1)	8/8 (3.3)
Hypospermia	0/8	-	0/8	0/8	0/8	8/8 (3.3)
Females						
Animals initially in study	8	8	8	8	8	8
Peripheral nerve						
Axon degeneration	0/8	-	-	-	0/8	8/8 (3.0)
Schwann cell degeneration	0/8	-	-	-	0/8	8/8 (3.0)
Spinal cord						
Lumbar axon degeneration	0/8	-	-	-	0/4	8/8 (1.8)
Skeletal muscle						
Atrophy	0/8	-	-	-	0/8	7/8 (2.0)
Urinary bladder						
Dilatation	0/8	-	-	-	-	6/8 (2.3)
Spleen						
Congestion	0/8	-	-	-	0/8	8/8 (2.8)
Pigmentation	0/8	-	-	-	0/8	8/8 (2.8)
Bone marrow						
Erythroid cell hyperplasia	0/8	-	-	-	0/8	8/8 (2.0)
Uterus						
Anestrus	0/8	-	-	-	0/8	8/8

[a] Data are reported as the number of lesions per number of rats examined microscopically. The average severity is given in parentheses. Severity was scored as: 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked.
[b] not examined

TABLE 10
Incidence of Nonneoplastic Lesions in Rats in the 3-Month Feed Study of Acrylamide[a]

	0 mg/kg	7.4 mg/kg	18.5 mg/kg	37 mg/kg	74 mg/kg	185 mg/kg
Males						
Animals initially in study	8	8	8	8	8	8
Peripheral nerve						
Axon degeneration	0/8	-[b]	-	-	0/8	8/8 (1.0)
Schwann cell degeneration	0/8	-	-	-	0/8	8/8 (1.0)
Spinal cord						
Lumbar axon degeneration	0/8	-	-	-	-	0/8
Skeletal muscle						
Atrophy	0/8	-	-	-	0/8	8/8 (1.0)
Urinary bladder						
Dilatation	1/8 (2.0)	-	-	-	-	3/8 (3.0)
Spleen						
Congestion	0/8	-	-	-	-	0/8
Pigmentation	0/8	-	-	-	-	2/8 (3.0)
Bone marrow						
Erythroid cell hyperplasia	0/8	-	-	-	-	0/8
Testes						
Germinal epithelium degeneration	0/8	2/8 (1.5)	2/8 (1.0)	4/8 (1.3)	7/8 (1.0)	8/8 (2.1)
Epididymis						
Exfoliated germ cell	0/8	1/8 (2.0)	0/8	3/8 (1.7)	8/8 (1.4)	8/8 (2.5)
Hypospermia	0/8	0/8	0/8	0/8	0/8	4/8 (2.5)
Females						
Animals initially in study	8	8	8	8	8	8
Peripheral nerve						
Axon degeneration	0/8	-	-	-	2/8 (1.0)	8/8 (1.0)
Schwann cell degeneration	0/8	-	-	-	2/8 (1.0)	8/8 (1.0)
Spinal cord						
Lumbar axon degeneration	0/8	-	-	-	-	1/8 (1.0)
Skeletal muscle						
Atrophy	0/8	-	-	-	0/8	7/8 (1.0)
Urinary bladder						
Dilatation	0/8	-	-	-	-	3/7 (2.3)
Spleen						
Congestion	0/8	-	-	-	-	0/8
Pigmentation	0/8	-	-	-	-	0/8
Bone marrow						
Erythroid cell hyperplasia	0/8	-	-	-	-	0/8
Uterus						
Anestrus	0/8	-	-	-	-	0/8

[a] Data are reported as the number of lesions per number of rats examined microscopically. The average severity is given in parentheses. Severity was scored as: 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked.
[b] Not examined.

Exposure Concentration Selection Rationale for 2-Year Drinking Water Study: In the 13-week study, 3.52 mM acrylamide in the drinking water caused decreased body weight, hind-leg paralysis, urinary bladder dilatation, radiculoneuropathy (typically accompanied by skeletal muscle atrophy), increased hemosiderin pigment in the spleen, and hyperplasia of erythrocytic precursors in bone marrow. Testicular germinal epithelium degeneration occurred in all male rats administered 1.41 and 3.52 mM acrylamide and in five of eight males administered 0.70 mM acrylamide. Four of eight females displayed hind-leg paralysis in the 1.41 mM acrylamide drinking water group. Based upon these observations, a high dose of 0.70 mM acrylamide (50 ppm acrylamide) was selected for the 2-year drinking water study, with the remaining doses being 0.0, 0.0875, 0.175, and 0.35 mM acrylamide (0, 6.25, 12.5, and 25 ppm acrylamide). The 0.35 mM acrylamide treatment was projected to produce a dose rate of exposure similar to that used in the Johnson *et al*. (1986) and Friedman *et al*. (1995) bioassays.

As noted in the Study Rationale, one objective of this study was to compare the induction of tumors by acrylamide with that of its metabolite glycidamide, as a function of dose, in F344/N rats. In the range finding and subchronic studies in F344/N rats, acrylamide gave similar responses when administered in the diet and in the drinking water. Since glycidamide rapidly decomposes when mixed in the diet (greater than 30% decomposition in one day; D.R. Doerge and N.C. Twaddle, unpublished observation) only drinking water exposures were used in the 2-year study phase of the experiment to allow a direct comparison between the responses induced by acrylamide with those induced by glycidamide.

2-Year Study

Survival and Cause of Death

Acrylamide in the drinking water had no effect upon the survival of male F344/N rats but caused a dose-related decreasing trend in survival in female F344/N rats (Table 11 and Figure 7). Female F344/N rats administered 0.175, 0.35, and 0.70 mM acrylamide had a decreased survival compared to control female rats (Figure 7 and Table 11), with 27, 25, and 35, respectively, of the rats being removed before the scheduled terminal sacrifice due to moribundity or death. The primary cause (greater than or equal to 70%) for the early removal or death of these rats was neoplasms, including mononuclear cell leukemia, mammary gland fibroadenoma, clitoral gland adenoma or carcinoma, pituitary gland adenoma or carcinoma, and Zymbal's gland squamous cell carcinoma.

TABLE 11
Survival and Disposition of Rats in the 2-Year Drinking Water Study of Acrylamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Male					
Animals initially in study	48	48	48	48	48
Moribund	23	29	27	30	34
Natural deaths	8	5	2	2	5
Animals surviving to study termination[a]	17	14	19	16	9
Percent probability of survival at end of study[b]	35	29	42	33	19
Mean survival (weeks)[c]	93.1	91.3	95.7	95.5	90.6
Survival analysis[d]	P = 0.065	P = 0.576	P = 0.534	P = 0.890	P = 0.080
Female					
Animals initially in study	48	48	48	48	48
Moribund	10	18	24	20	33
Natural deaths	4	2	3	5	2
Animals surviving to study termination[a]	34	28	21	23	13
Percent probability of survival at end of study[b]	71	58	44	48	27
Mean survival (weeks)[c]	100.0	98.5	96.8	92.3	89.2
Survival analysis[d]	P < 0.001	P = 0.222	P = 0.013	P = 0.015	P <0.001

[a] Censored from the survival analyses.
[b] Kaplan-Meier survival estimates.
[c] Mean of all deaths (censored and uncensored).
[d] The result of the life table trend test (Tarone, 1975) is in the 0.0 μM acrylamide column, and the results of the life table pairwise comparisons (Cox, 1972) with the 0.0 μM acrylamide are in the treatment group columns.

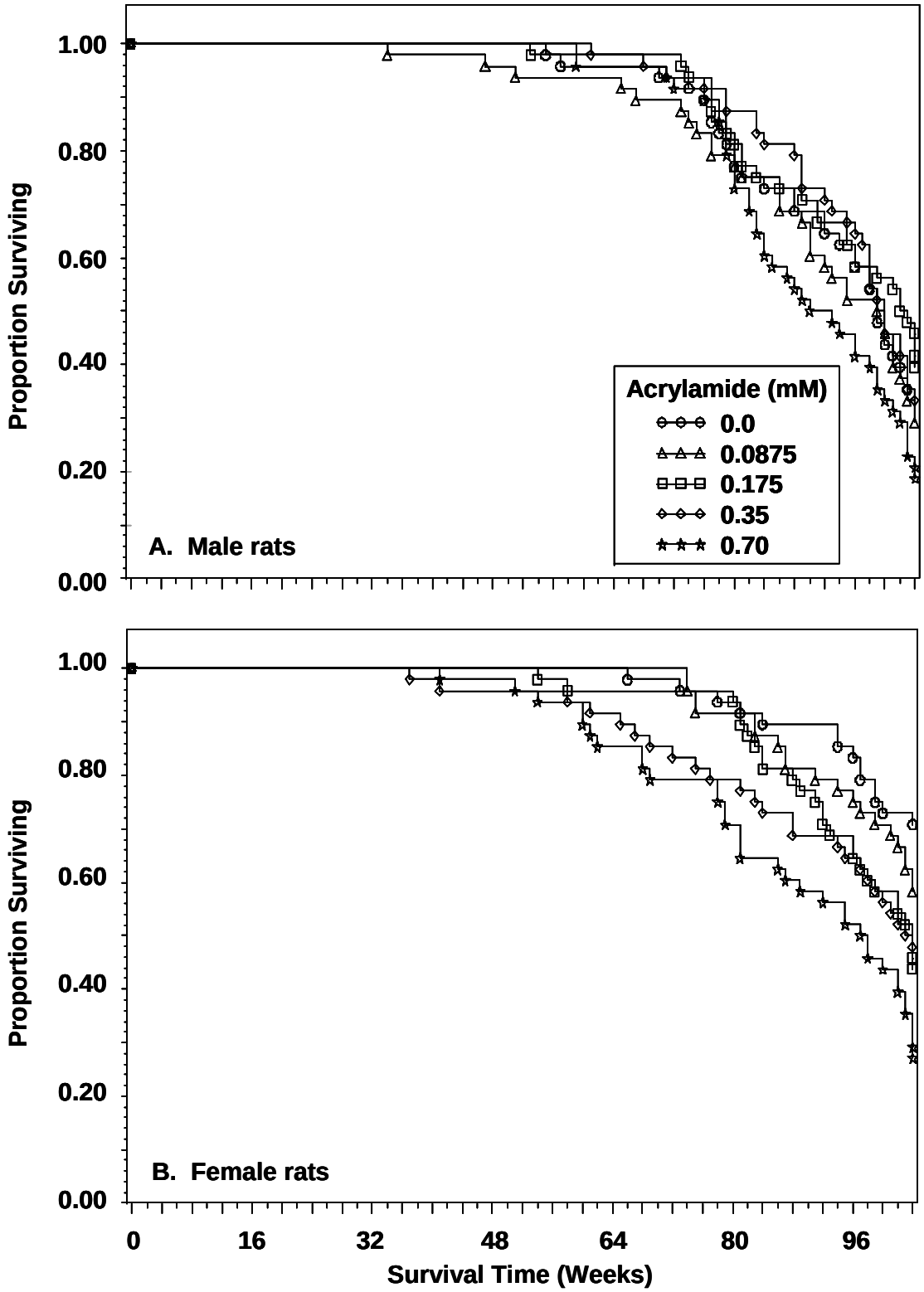


FIGURE 7
Kaplan-Meier Survival Curves for Male and Female Rats Administered Acrylamide in Drinking Water for 2 Years

Body Weights and Feed and Water Consumption

Acrylamide in the drinking water caused significant dose-related decreasing trends in body weight in male F344/N rats at weeks 48, 60, 64, and 80 to 104 (Figure 8 and Table 12). In female rats, there were significant dose-related decreasing trends in body weight beginning at 8 weeks (Figure 8 and Table 13). Treatment with 0.70 mM acrylamide resulted in significant decreases in body weight gain beginning at week 80 in the male rats and week 8 in the female rats. At the end of the 2 year period, the male rats administered 0.70 mM acrylamide weighed 86% of the control group; the female rats administered 0.70 mM acrylamide weighed 85% of the control group.

Acrylamide in the drinking water caused sporadic dose-related increasing trends in food consumption in male (Table G1) and female (Table G2) F344/N rats.

Acrylamide in the drinking water did not affect water consumption in male F344/N rats (Table 14). In female F344/N rats, acrylamide in the drinking water caused a dose-related increasing trend in water consumption beginning at week 68 (Table 15). Water consumption in the 0.70 mM acrylamide group of female F344/N rats was significantly increased compared to the control group at week 72 and weeks 84 to 104 (Table 15).

The mean acrylamide exposure for the F344/N rats, calculated at 4 week intervals, is presented in Figure 9 and Tables 14 and 15. The mean amount of acrylamide consumed by male F344/N rats for the entire 2 year study was 0.33, 0.66, 1.32, and 2.71 mg acrylamide per kg body weight per day for the 0.0875, 0.175, 0.35, and 0.70 mM acrylamide (6.25, 12.5, 25, and 50 ppm acrylamide) dose groups, respectively. The corresponding values for female rats were 0.44, 0.88, 1.84, and 4.02 mg acrylamide per kg body weight per day.

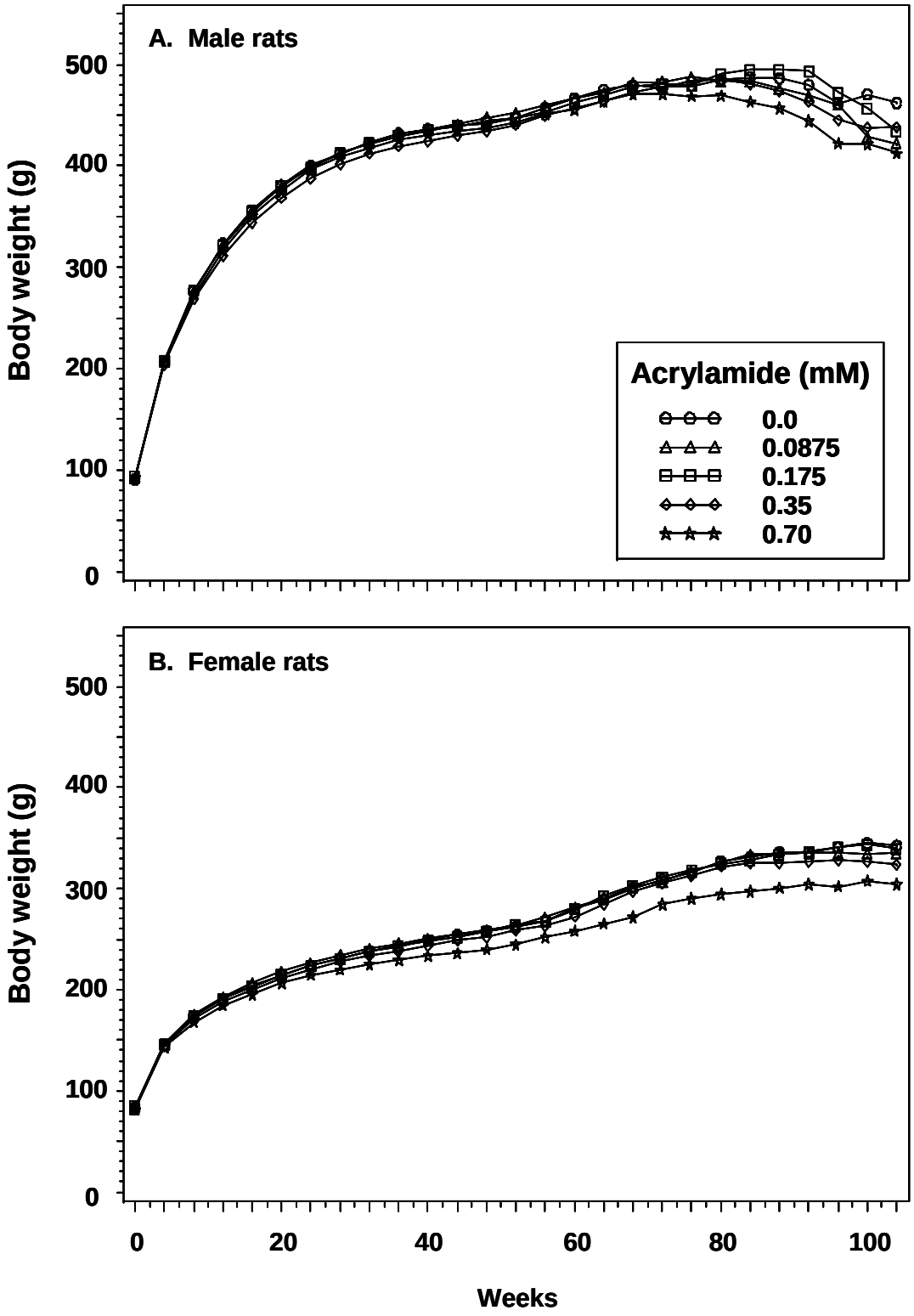


FIGURE 8
Growth Curves for Male and Female Rats Administered Acrylamide in Drinking Water for 2 Years

TABLE 12
Mean Body Weights[a] and Survival of Male Rats in the 2-Year Drinking Water Study of Acrylamide

Weeks on Study	0 mM		0.0875 mM			0.175 mM			0.35 mM			0.70 mM		
	Mean Wt. (g)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors
4	206.1	48	206.6	100.2	48	207.4	100.6	48	203.5	98.7	48	206.0	100.0	48
8	276.8	48	276.7	100.0	48	277.5	100.2	48	268.5	97.0	48	272.5	98.4	48
12	323.4	48	322.8	99.8	48	321.5	99.4	48	311.3*	96.2	48	317.4	98.1	48
16	356.8	48	355.8	99.7	48	354.2	99.3	48	344.2*	96.5	48	351.3	98.4	48
20	381.1	48	379.5	99.6	48	380.2	99.8	48	368.4*	96.7	48	375.3	98.5	48
24	399.8	48	399.3	99.9	48	397.7	99.5	48	387.3*	96.9	48	396.0	99.0	48
28	412.4	48	412.3	100.0	48	412.7	100.1	48	401.4	97.3	48	409.1	99.2	48
32	422.6	48	423.2	100.1	48	422.2	99.9	48	412.4	97.6	48	417.4	98.8	48
36	432.0	48	431.7	99.9	47	429.4	99.4	48	419.3*	97.1	48	426.1	98.6	48
40	436.3	48	435.8	99.9	47	434.9	99.7	48	424.4*	97.3	48	430.8	98.8	48
44	439.0	48	441.1	100.5	47	440.1	100.2	48	430.2	98.0	48	434.6	99.0	48
48	444.0*	48	447.7	100.8	46	442.1	99.6	48	433.9	97.7	48	437.7	98.6	48
52	446.9	48	452.2	101.2	45	447.3	100.1	48	440.6	98.6	48	442.2	99.0	48
56	457.1	47	458.8	100.4	45	453.4	99.2	47	449.5	98.3	48	450.7	98.6	48
60	466.0*	46	466.1	100.0	45	462.6	99.3	47	456.8	98.0	48	454.4	97.5	46
64	474.6*	46	472.4	99.5	45	470.0	99.0	47	464.4	97.9	47	463.3	97.6	46
68	478.8	46	478.4	99.9	43	478.3	99.9	47	472.3	98.6	47	470.0	98.2	46
72	476.9	45	479.8	100.6	43	481.1	100.9	47	476.4	99.9	45	470.9	98.7	45
76	480.1	44	480.8	100.1	40	477.7	99.5	45	476.1	99.2	45	467.7	97.4	44
80	482.6*	39	478.8	99.2	38	483.1	100.1	40	480.0	99.5	42	465.3*	96.4	38
84	480.3*	36	478.3	99.6	36	483.2	100.6	36	477.1	99.3	40	456.1*	95.0	31
88	476.9*	35	468.4	98.2	33	484.1	101.5	35	468.0	98.1	39	445.2*	93.4	27
92	465.0*	33	453.1	97.5	29	484.9	104.3	32	451.3	97.1	35	429.4*	92.3	24
96	436.2*	30	431.5	98.9	25	463.5	106.3	30	427.7	98.0	32	401.9*	92.1	22
100	435.5*	23	401.8	92.3	24	443.5	101.9	27	407.2	93.5	25	385.3*	88.5	17
104	430.2*	17	380.4*	88.4	16	415.0	96.5	23	398.3	92.6	17	368.6*	85.7	11
Mean for Weeks														
4-104	423.7		419.7			424.9			413.5			409.4		

[a] An * in the 0.0 mM acrylamide column indicates a significant dose-related trend ($p<0.05$); an * in the treatment column indicates a significant ($p<0.05$) pairwise comparison of the dose group to the 0.0 mM acrylamide group as determined by Dunnett's test.

TABLE 13
Mean Body Weights[a] and Survival of Female Rats in the 2-Year Drinking Water Study of Acrylamide

Weeks on Study	0 mM		0.0875 mM			0.175 mM			0.35 mM			0.70 mM		
	Mean Wt. (g)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors
4	145.8	48	146.2	100.2	48	146.1	100.2	48	144.4	99.0	48	143.1	98.1	48
8	173.9*	48	175.8	101.1	48	174.0	100.1	48	171.8	98.8	48	168.0*	96.6	48
12	191.4*	48	193.0	100.8	48	191.4	100.0	48	188.1	98.2	48	184.8*	96.5	48
16	202.8*	48	206.5	101.8	48	204.0	100.6	48	200.3	98.7	48	195.4*	96.3	48
20	214.3*	48	218.0	101.7	48	214.5	100.1	48	211.6	98.7	48	206.5*	96.4	48
24	223.9*	48	226.9	101.3	48	223.6	99.9	48	220.3	98.4	48	214.6*	95.8	48
28	231.4*	48	234.5	101.3	48	230.9	99.8	48	227.8	98.4	48	220.1*	95.1	48
32	237.7*	48	241.0	101.4	48	237.7	100.0	48	233.4	98.2	48	225.2*	94.8	48
36	242.5*	48	245.7	101.3	48	243.1	100.2	48	237.4	97.9	48	229.3*	94.6	48
40	249.6*	48	250.2	100.3	48	248.4	99.5	48	242.4	97.1	47	233.3*	93.5	48
44	254.5*	48	255.0	100.2	48	252.4	99.2	48	247.9	97.4	46	236.2*	92.8	47
48	258.2*	48	259.0	100.3	48	257.2	99.6	48	250.4	97.0	46	239.9*	92.9	47
52	261.7*	48	263.6	100.7	48	265.1	101.3	48	257.9	98.5	46	243.8*	93.1	46
56	268.3*	48	271.4	101.1	48	268.3	100.0	47	261.6	97.5	46	249.8*	93.1	45
60	279.9*	48	281.5	100.6	48	279.8	100.0	46	270.5	96.6	45	256.1*	91.5	45
64	289.4*	48	290.9	100.5	48	293.4	101.4	46	281.9	97.4	44	262.6*	90.7	41
68	299.9*	47	301.7	100.6	48	303.0	101.0	46	292.6	97.6	42	269.0*	89.7	41
72	308.5*	47	307.6	99.7	48	311.7	101.0	46	300.2	97.3	41	279.4*	90.6	38
76	315.1*	46	314.8	99.9	44	319.0	101.2	46	308.7	98.0	39	285.4*	90.6	38
80	325.2*	45	324.4	99.7	44	324.9	99.9	46	316.2	97.2	38	287.9*	88.5	34
84	329.7*	44	331.6	100.6	42	327.2	99.2	41	320.9	97.4	36	290.9*	88.2	31
88	334.1*	43	331.9	99.4	39	328.9	98.5	39	321.6	96.3	35	295.4*	88.4	29
92	333.4*	43	333.9	100.1	38	325.9	97.8	36	321.8	96.5	33	295.9*	88.7	28
96	336.8*	41	331.2	98.3	37	325.5	96.7	33	318.4	94.5	31	293.2*	87.1	25
100	338.4*	36	327.2	96.7	34	325.2	96.1	28	314.6*	92.9	28	294.9*	87.1	22
104	337.6*	35	323.6	95.9	30	319.9	94.8	25	305.5*	90.5	24	285.6*	84.6	17
Mean for Weeks														
4-104	268.6		268.7			267.0			260.3			245.6		

[a] An * in the 0.0 mM acrylamide column indicates a significant dose-related trend ($p<0.05$); an * in the treatment column indicates a significant ($p<0.05$) pairwise comparison of the dose group to the 0.0 mM acrylamide group as determined by Dunnett's test.

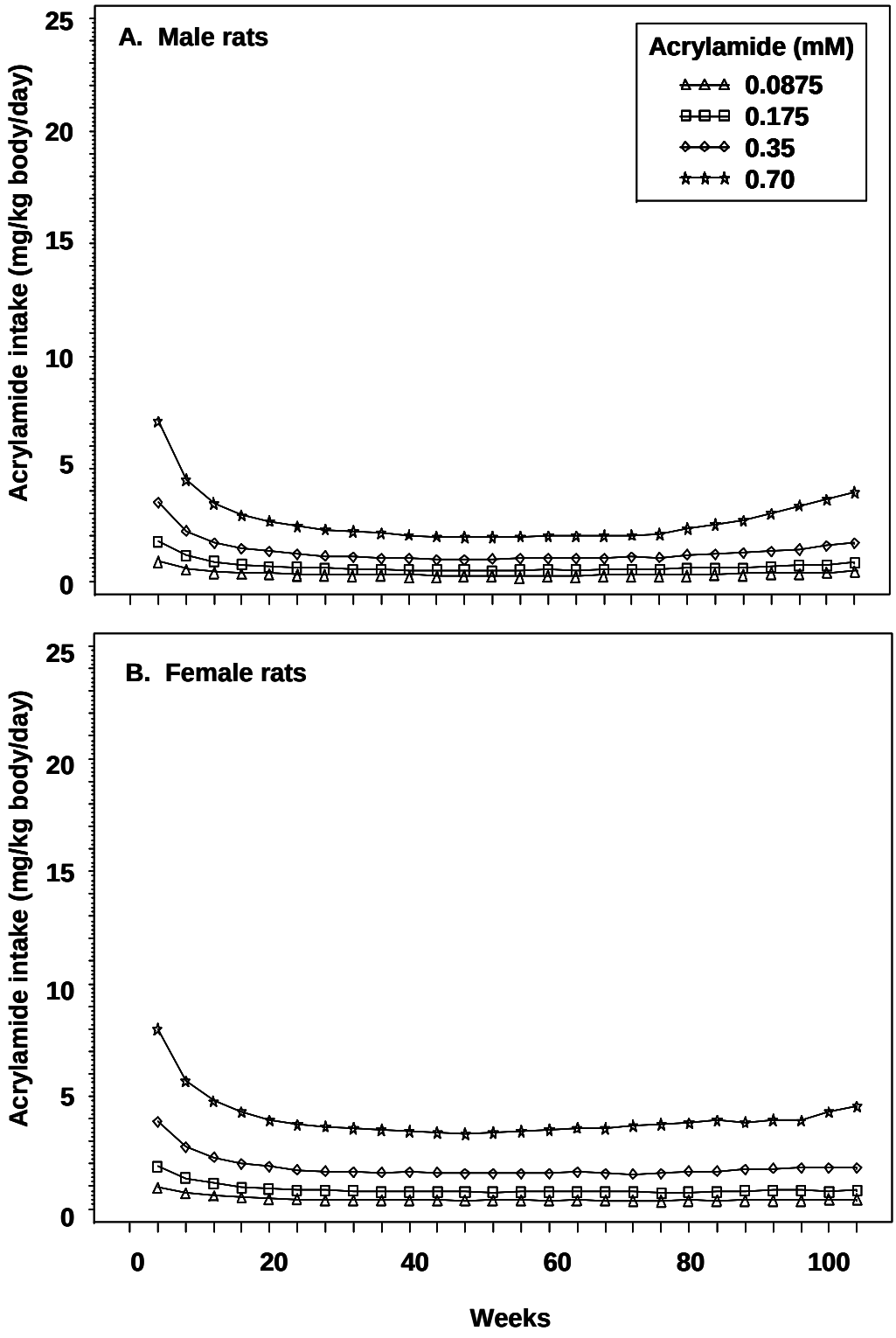


FIGURE 9
Acrylamide Intake in Male and Female Rats Administered Acrylamide in Drinking Water for 2 Years

TABLE 14
Water and Acrylamide Consumption by Male Rats in the 2-Year Drinking Water Study of Acrylamide

Week[a]	0 mM		0.0875 mM			0.175 mM			0.35 mM			0.70 mM		
	Water (g/day)	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose[b]	Water (g/day)	Body Weight (g)	Dose	Water (g/day)	Body Weight (g)	Dose	Water (g/day)	Body Weight (g)	Dose
4	21.4	206.1	21.6	206.6	0.86	21.8	207.4	1.75	21.1	203.5	3.49	22.0	206.0	7.10
8	21.9	276.8	21.6	276.7	0.55	22.0	277.5	1.13	21.2	268.5	2.23	21.7	272.5	4.52
12	20.8	323.4	20.4	322.8	0.42	20.9	321.5	0.87	19.6	311.3*	1.69	20.4	317.4	3.44
16	20.0	356.8	19.6	355.8	0.36	20.1	354.2	0.74	19.2	344.2*	1.46	19.6	351.3	2.93
20	19.4	381.1	18.8	379.5	0.32	19.6	380.2	0.66	18.9	368.4*	1.32	19.5	375.3	2.67
24	18.6	399.8	18.2	399.3	0.29	19.1	397.7	0.61	18.4	387.3*	1.21	18.9	396.0	2.44
28	18.3	412.4	18.0	412.3	0.28	18.6	412.7	0.57	17.8	401.4	1.12	18.5	409.1	2.30
32	17.8	422.6	17.9	423.2	0.27	18.0	422.2	0.54	17.5	412.4	1.07	18.4	417.4	2.22
36	18.0	432.0	17.9	431.7	0.26	17.8	429.4	0.52	17.5	419.3*	1.04	18.0	426.1	2.13
40	17.5	436.3	17.8	435.8	0.25	17.3	434.9	0.50	17.2	424.4*	1.01	17.6	430.8	2.05
44	17.2	439.0	17.3	441.1	0.24	17.2	440.1	0.49	16.8	430.2	0.98	17.3	434.6	1.99
48	17.2	444.0*[c]	17.5	447.7	0.25	17.2	442.1	0.49	17.0	433.9	0.98	17.3	437.7	1.97
52	17.2	446.9	17.8	452.2	0.25	17.2	447.3	0.48	17.5	440.6	1.00	17.4	442.2	1.97
56	17.9	457.1	18.5	458.8	0.25	17.8	453.4	0.49	18.1	449.5	1.01	17.7	450.7	1.98
60	18.6	466.0*	18.6	466.1	0.25	18.6	462.6	0.51	18.3	456.8	1.01	18.1	454.4	2.01
64	18.9	474.6*	19.4	472.4	0.25	18.8	470.0	0.50	19.1	464.4	1.04	18.8	463.3	2.00
68	19.2	478.8	19.8	478.4	0.26	19.3	478.3	0.51	19.7	472.3	1.04	19.4	470.0	2.04
72	19.6	476.9	20.2	479.8	0.26	19.6	481.1	0.51	20.3	476.4	1.07	19.6	470.9	2.04
76	20.3	480.1	20.7	480.8	0.27	20.0	477.7	0.52	20.4	476.1	1.06	20.0	467.7	2.10
80	20.8	482.6*	22.3	478.8	0.29	20.9	483.1	0.58	22.5	480.0	1.18	21.8	465.3*	2.33
84	22.4	480.3*	23.7	478.3	0.30	23.6	483.2	0.60	23.4	477.1	1.23	23.3	456.1*	2.51
88	23.4	476.9*	24.2	468.4	0.32	24.6	484.1	0.61	24.1	468.0	1.26	25.0	445.2*	2.68
92	24.6	465.0*	26.6	453.1	0.36	25.5	484.9	0.64	24.5	451.3	1.31	27.3	429.4*	3.00
96	25.5	436.2*	24.1	431.5	0.34	27.5	463.5	0.73	24.8	427.7	1.42	28.7	401.9*	3.35
100	28.0	435.5*	24.7	401.8	0.37	29.2	443.5	0.72	26.1	407.2	1.60	29.8	385.3*	3.62
104	31.8	430.2*	27.8	380.4*	0.45	31.2	415.0	0.85	27.0	398.3	1.69	30.9	368.6*	3.94
Mean for weeks														
4-104	20.6	423.7	20.6	419.7	0.33	20.9	424.9	0.66	20.3	413.5	1.32	21.0	409.4	2.71

[a] Week indicates the last week of a four-week interval of daily water consumption, measured weekly by cage.
[b] Dose is expressed as the mean value measured in mg/kg body weight/day.
[c] An * in the 0.0 mM acrylamide column indicates a significant dose-related trend ($p<0.05$); an * in the treatment column indicates a significant ($p<0.05$) pairwise comparison of the dose group to the 0.0 mM acrylamide group as determined by Dunnett's test.

TABLE 15
Water and Acrylamide Consumption by Female Rats in the 2-Year Drinking Water Study of Acrylamide

Week[a]	0 mM		0.0875 mM			0.175 mM			0.35 mM			0.70 mM		
	Water (g/day)	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose[b]	Water (g/day)	Body Weight (g)	Dose	Water (g/day)	Body Weight (g)	Dose	Water (g/day)	Body Weight (g)	Dose
4	18.8	145.8	19.1	146.2	0.98	18.7	146.1	1.90	18.9	144.4	3.89	19.1	143.1	7.98
8	18.0	173.9*[c]	18.6	175.8	0.72	17.8	174.0	1.37	17.7	171.8	2.75	17.8	168.0*	5.66
12	16.5	191.4*	17.1	193.0	0.58	16.7	191.4	1.13	16.6	188.1	2.30	16.9	184.8*	4.79
16	15.7	202.8*	16.2	206.5	0.50	15.6	204.0	0.98	15.8	200.3	2.02	16.4	195.4*	4.30
20	15.4	214.3*	16.2	218.0	0.48	15.3	214.5	0.91	15.7	211.6	1.90	15.9	206.5*	3.93
24	15.2	223.9*	15.6	226.9	0.44	15.0	223.6	0.85	15.1	220.3	1.74	15.8	214.6*	3.74
28	15.2*	231.4*	15.4	234.5	0.42	15.1	230.9	0.82	15.1	227.8	1.68	16.0	220.1*	3.66
32	15.3	237.7*	15.6	241.0	0.41	15.2	237.7	0.81	15.3	233.4	1.65	16.0	225.2*	3.56
36	15.6	242.5*	15.7	245.7	0.40	15.1	243.1	0.79	15.3	237.4	1.62	16.0	229.3*	3.50
40	15.6	249.6*	15.5	250.2	0.39	15.3	248.4	0.78	15.6	242.4	1.63	16.0	233.3*	3.44
44	15.6	254.5*	15.5	255.0	0.38	15.2	252.4	0.76	15.8	247.9	1.61	15.8	236.2*	3.38
48	15.7	258.2*	15.5	259.0	0.37	15.4	257.2	0.75	15.9	250.4	1.58	15.8	239.9*	3.32
52	16.1	261.7*	15.9	263.6	0.38	15.7	265.1	0.75	16.0	257.9	1.56	16.4	243.8*	3.39
56	16.7	268.3*	16.4	271.4	0.38	16.0	268.3	0.76	16.5	261.6	1.58	17.0	249.8*	3.45
60	17.1	279.9*	16.8	281.5	0.38	17.1	279.8	0.78	17.1	270.5	1.60	17.7	256.1*	3.50
64	17.5	289.4*	17.4	290.9	0.38	18.1	293.4	0.79	18.1	281.9	1.62	18.6	262.6*	3.60
68	18.0*	299.9*	17.9	301.7	0.38	18.0	303.0	0.75	18.3	292.6	1.59	19.5	269.0*	3.57
72	18.2*	308.5*	18.2	307.6	0.37	18.9	311.7	0.76	18.7	300.2	1.56	20.6*	279.4*	3.72
76	19.1*	315.1*	17.8	314.8	0.36	18.6	319.0	0.73	19.4	308.7	1.58	21.7	285.4*	3.76
80	19.9*	325.2*	19.1	324.4	0.38	19.2	324.9	0.74	21.4	316.2	1.68	22.1	287.9*	3.80
84	19.6*	329.7*	19.4	331.6	0.38	20.3	327.2	0.79	21.6	320.9	1.68	23.7*	290.9*	3.93
88	20.3*	334.1*	19.4	331.9	0.39	21.6	328.9	0.80	22.8*	321.6	1.76	23.3*	295.4*	3.84
92	21.4*	333.4*	21.0	333.9	0.40	22.1	325.9	0.82	23.7	321.8	1.80	24.6*	295.9*	3.96
96	21.4*	336.8*	21.8	331.2	0.42	22.9	325.5	0.85	23.5	318.4	1.82	24.4*	293.2*	3.92
100	21.7*	338.4*	22.9	327.2	0.44	21.5	325.2	0.78	24.1	314.6*	1.85	26.1*	294.9*	4.30
104	22.1*	337.6*	22.5	323.6	0.44	22.0	319.9	0.84	24.1	305.5*	1.82	28.3*	285.6*	4.55
Mean for weeks														
4-104	17.8	268.6	17.8	268.7	0.44	17.8	267.0	0.88	18.4	260.3	1.84	19.3	245.6	4.02

[a] Week indicates the last week of a four-week interval of daily water consumption, measured weekly by cage.
[b] Dose is expressed as the mean value measured in mg/kg body weight/day.
[c] In the 0.0 mM acrylamide column "*" indicates a significant trend ($p < 0.05$); in the treatment column "*" indicates a significant ($p < 0.05$) pairwise comparison of the dose group to the 0.0 mM acrylamide group as determined by Dunnett's test.

Neoplastic Findings

The administration of acrylamide in the drinking water resulted in thyroid gland neoplasms in both sexes of F334/N rats. In male rats, there was a dose-related increase in thyroid gland follicular cell adenoma, follicular cell carcinoma, and combined follicular cell adenoma or carcinoma, with the incidence of follicular cell carcinoma and combined follicular cell adenoma or carcinoma being significant at 0.70 mM acrylamide (Tables 16 and A2). In female rats, thyroid gland follicular cell carcinoma and combined follicular cell adenoma or carcinoma showed a dose-related increasing trend, with the incidence of combined follicular cell adenoma or carcinoma of being significant at 0.70 mM acrylamide (Tables 17 and B2).

Morphologically, both follicular cell adenomas and follicular cell carcinomas were typical of spontaneous thyroid gland follicular neoplasms in F344/N rats. Adenomas were small circumscribed solitary lesions that slightly compressed adjacent parenchyma. The well-differentiated follicular cells were usually arranged in a single layer on the basement membrane. Adenomas were arranged in either a follicular or a papillary pattern that sometimes included cystic structures. Follicular cell carcinomas were larger masses without definite boundaries and with disorganized growth patterns. The cells were pleomorphic, sometimes atypical, and were often piled in multiple layers or arranged in solid clusters or sheets. Invasion of the thyroid gland capsule or blood vessels occurred occasionally.

Dose-related increases in malignant schwannoma in the heart occurred in both sexes of F344/N rats (Tables 16, 17, A2, and B2), with the incidence being significant in male rats administered 0.70 mM acrylamide. The microscopic morphology of schwannomas in the heart was similar in lesions located in either subendocardial or intramural locations and consisted of spindeloid cells with fusiform granular to hyperchromatic nuclei and pale indistinct cytoplasm. The neoplastic cells were arranged in either discreet foci or more commonly infiltrated around and between adjacent myocardial fibers. The designation of malignancy was based primarily on the infiltrative characteristic.

Consumption of acrylamide in the drinking water was associated with development of malignant mesothelioma on membranes surrounding the epididymis and on testicular tunics in male rats (Tables 16 and A2). Malignant mesothelioma was more commonly observed in the epididymis than on the testes, and all rats having mesothelioma on the testicular tunics also had this neoplasm on the epididymis. Compared to the control group, the incidence of malignant mesothelioma was significantly increased in the epididymis and combined testes and epididymis in male rats administered 0.70 mM acrylamide (Tables 16 and A2). Malignant mesothelioma was also observed in many other pelvic and abdominal organs in several animals but the prevalence of this neoplasm in locations other than epididymis and testis did not indicate a relationship to treatment. Many may have originated on the epididymis and spread to the other locations. Microscopically, malignant mesotheliomas in the epididymis and testicular tunics were characterized by complex papillary surface growths of one to several layers of polyhedral to cuboidal mesothelial cells on pedunculated fibrovascular stalks. The neoplastic cells had either abundant weakly eosinophilic cytoplasm and ovoid nuclei with one or more nucleoli or scanty cytoplasm and numerous small basophilic nuclei.

In male rats, there was a dose-related increase in pancreatic islet adenoma and the incidence was significantly increased in the 0.70 mM group (Tables 16 and A2). Microscopically islet cell adenomas resembled normal pancreatic islets but were at least twice the size of normal islets with some compression of adjacent acinar tissue. A single islet cell carcinoma was observed in the 0.35 mM group. The islet cell carcinoma was larger than the islet cell adenomas and the component cells were less differentiated.

In female rats, the prevalence of fibroadenomas in the mammary gland was related to acrylamide treatment, with the incidence in the 0.175, 0.35, and 0.70 mM groups being significantly increased compared to the control group (Tables 17 and B2). Microscopically mammary fibroadenomas were characterized by variable amounts of uniform well-differentiated glandular or epithelium embedded in dense mature fibrous connective tissue. The neoplasms were well-circumscribed and well-demarcated from adjacent tissue.

Four types of epithelial neoplasms (adenomas, carcinomas, squamous cell papillomas, and squamous cell carcinomas) were observed in the clitoral glands of female rats. Clitoral gland adenomas were circumscribed masses that compressed adjacent tissue. Some of the acinar structure of the normal gland was retained in adenomas but the acini were usually ill defined and varied in size. The component cells were well differentiated and retained varied numbers of the eosinophilic granules that are typical in normal glands. Clitoral gland carcinomas were usually larger than adenomas and the borders of these neoplasms were irregular and indistinct. Acinar arrangement was usually not present in carcinomas;

instead, the neoplastic cells were arranged in irregular lobules, cords, or sheets. The degree of cellular anaplasia varied. Usually there were few eosinophilic cytoplasmic granules or they were completely absent. In many cases, extensive inflammation and fibrosis were associated with carcinomas. Fibrosis resulting from chronic inflammation was difficult to differentiate from the fibrous stroma of some carcinomas. The morphology of clitoral gland squamous cell papillomas consisted of papillae of well-differentiated squamous cells overlying fibrovascular cores. Clitoral gland squamous cell carcinomas were usually larger and were characterized by nests and sheets of squamous cells with varied individual cell keratinization. In some cases, the squamous cell carcinomas appeared to be situated near glandular ducts but in other cases there was squamous differentiation within glandular carcinoma. The diagnosis of squamous cell carcinoma in clitoral gland was used only when the neoplasm was almost totally or at least predominately differentiated toward squamous cells and when the sebaceous differentiation was absent or minimal. Of these neoplasms, acrylamide administration resulted in a dose-related increase in clitoral gland carcinoma and squamous cell papilloma, with the incidence of clitoral gland carcinoma being significant in the 0.0875, 0.175, and 0.70 mM groups (Tables 17 and B2).

In female rats, the drinking water administration of acrylamide was associated with a dose-related increase in oral mucosa squamous cell papilloma and combined oral mucosa or tongue squamous cell papilloma or carcinoma, with the incidence being significantly increased in the 0.70 mM group (Tables 17 and B2). Several of the oral squamous cell neoplasms originated in the tongue. Other locations in the oral cavity, most commonly the hard palate, were affected more often. Microscopically, the oral squamous cell papillomas were elevated nodules or masses consisting of several layers of well-differentiated squamous cells covering multiple projections of a fibrovascular core. The squamous cell carcinomas on the tongue were gross lesions situated on the dorsum of the tongue. Microscopic examination revealed that they consisted of atypical squamous cells that invaded tissues underlying the epithelium.

Drinking water administration of acrylamide was also associated with a dose-related increase in mesenchymal skin tumors (fibroma, fibrosarcoma, or sarcoma) in female F344/N rats, with the incidence being significant in the 0.70 mM group (Tables 17 and B2). Female F344/N rats also had significant dose-related increasing trend in liver hepatocellular adenoma (Tables 17 and B2).

During the neuropathology review, a few proliferative glial cell lesions, including several astrocytomas, one glioma, and focal gliosis in the brain or spinal cord were observed in both sexes of control and acrylamide-treated F344/N rats. Focal gliosis occurred in the brains of one male and three female F344/N rats. These were small irregular lesions, less than 1 mm in diameter, that lacked a discrete boundary. The cells had round to ovoid nuclei and usually variable amounts of cytoplasm that were either not apparent, fusiform, or polygonal. Occasionally, there was cuffing around vessels. Neither the astrocytomas nor gliosis showed dose-related trends or statistically significant incidences in either male or female F344/N rats.

TABLE 16
Statistical Analysis of Selected Neoplasms in Male Rats in the 2-Year Drinking Water Study of Acrylamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Epididymis: Malignant Mesothelioma					
Overall rate[a]	2/48 (4%)	2/48 (4%)	1/48 (2%)	5/48 (10)%	8/48 (17%)
Adjusted rate[b]	5.5%	5.8%	2.7%	13.1%	22.9%
Terminal rate[c]	1/17 (6%)	0/14 (0%)	0/19 (19%)	3/16 (19%)	1/9 (11%)
First incidence (days)[d]	533	557	690	620	500
Poly-3 test[e]	P=0.002	P=0.679	P=0.489N	P=0.232	P=0.034
Testes: Malignant Mesothelioma					
Overall rate	1/48 (2%)	2/48 (4%)	1/48 (2%)	1/48 (2)%	5/48 (10%)
Adjusted rate	2.7%	5.8%	2.7%	2.7%	14.5%
Terminal rate	0/17 (0%)	0/14 (0%)	0/19 (0%)	0/16 (0%)	0/9 (0%)
First incidence (days)	533	557	690	691	500
Poly-3 test	P=0.030	P=0.484	P=0.755N	P=0.753N	P=0.085
Epididymis or Testes: Malignant Mesothelioma[f]					
Overall rate	2/48 (4%)	2/48 (4%)	1/48 (2%)	5/48 (10)%	8/48 (17%)
Adjusted rate	5.5%	5.8%	2.7%	13.1%	22.9%
Terminal rate	1/17 (6%)	0/14 (0%)	0/19 (0%)	3/16 (19%)	1/9 (11%)
First incidence (days)	533	557	690	620	500
Poly-3 test	P=0.002	P=0.679	P=0.489N	P=0.232	P=0.034
Heart: Malignant Schwannoma[g]					
Overall rate	1/48 (2%)	2/48 (4%)	3/48 (6%)	4/48 (8%)	6/48 (13%)
Adjusted rate	2.8%	5.9%	7.9%	10.3%	18.3%
Terminal rate	1/17 (6%)	2/14 (14%)	1/19 (5%)	1/16 (6%)	3/9 (33%)
First incidence (days)	737 (T)	737 (T)	537	495	556
Poly-3 test	P=0.015	P=0.483	P=0.328	P=0.201	P=0.040
Pancreatic Islets: Adenoma[h]					
Overall rate	1/46 (2%)	2/48 (4%)	4/48 (8%)	1/48 (2%)	6/48 (13%)
Adjusted rate	2.8%	5.8%	10.4%	2.7%	18.0%
Terminal rate	1/17 (6%)	1/14 (7%)	1/19 (5%)	1/16 (6%)	2/9 (22%)
First incidence (days)	737 (T)	599	564	737 (T)	569
Poly-3 test	P=0.034	P=0.493	P=0.203	P=0.747N	P=0.044
Pancreatic Islets: Carcinoma					
Overall rate	0/46 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)	0/48 (0%)
Pancreatic Islets: Adenoma or Carcinoma[h]					
Overall rate	1/46 (2%)	2/48 (4%)	4/48 (8%)	2/48 (4%)	6/48 (13%)
Adjusted rate	2.8%	5.8%	10.4%	5.3%	18.0%
Terminal rate	1/17 (6%)	1/14 (7%)	1/19 (5%)	2/16 (13%)	2/9 (22%)
First incidence (days)	737 (T)	599	564	737 (T)	569
Poly-3 test	P=0.030	P=0.493	P=0.203	P=0.522	P=0.044
Thyroid Gland: Follicular Cell Adenoma					
Overall rate	0/47 (0%)	1/48 (2%)	1/47 (2%)	1/48 (2%)	3/48 (6%)
Adjusted rate	0%	2.9%	2.7%	2.7%	9.2%
Terminal rate	0/17 (0%)	0/14 (0%)	0/19 (0%)	0/16 (0%)	1/9 (11%)
First incidence (days)	-	688	690	711	556
Poly-3 test	P=0.047	P=0.492	P=0.510	P=0.511	P=0.102
Thyroid Gland: Follicular Cell Carcinoma[i]					
Overall rate	1/47 (2%)	2/48 (4%)	3/47 (6%)	6/48 (13%)	6/48 (13%)
Adjusted rate	2.8%	5.8%	7.9%	15.8%	17.6%
Terminal rate	1/17 (6%)	0/14 (0%)	2/19 (11%)	3/16 (19%)	0/9 (0%)
First incidence (days)	737 (T)	630	537	679	569
Poly-3 test	P=0.013	P=0.489	P=0.326	P=0.063	P=0.045

TABLE 16
Statistical Analysis of Selected Neoplasms in Male Rats in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Thyroid Gland: Follicular Cell Adenoma or Carcinoma[j]					
Overall rate	1/47 (2%)	3/48 (6%)	4/47 (9%)	6/48 (13%)	9/48 (19%)
Adjusted rate	2.8%	8.7%	10.5%	15.8%	25.9%
Terminal rate	1/17 (6%)	0/14 (0%)	2/19 (11%)	3/16 (19%)	1/9 (11%)
First incidence (days)	737 (T)	630	537	679	556
Poly-3 test	P=0.002	P=0.294	P=0.196	P=0.063	P=0.005

[a] Number of animals with neoplasm per number of animals examined microscopically.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[c] Observed incidence at the terminal sacrifice.
[d] T indicates terminal sacrifice.
[e] Beneath the 0 mM acrylamide are the p values associated with the trend test. Beneath the treated group incidences are the p values corresponding to pairwise comparisons between the 0 mM acrylamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice. An N indicates a negative trend or decreased tumor incidence.
[f] The historical incidence of mesothelioma (all sites) in NCTR control male F344/N rats is 4.8% (range 3.3% to 6.4%; Table A3b).
[g] The historical incidence of malignant schwannoma of the heart in NCTR control male F344/N rats is 0.0% (Table A3c).
[h] The historical incidence of the pancreas islet adenoma in NCTR control male F344/N rats is 8.8% (range 4.2% to 13.1%; Table A3d).
[i] The historical incidence of thyroid gland follicular cell carcinoma in NCTR control male F344/N rats is 0.0% (Table A3a).
[j] The historical incidence of thyroid gland follicular cell adenoma or carcinoma in NCTR control male F344/N rats is 0.4% (range 0.0% to 2.1%; Table A3a).

TABLE 17
Statistical Analysis of Selected Neoplasms in Female Rats in the 2-Year Drinking Water Study of Acrylamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Clitoral Gland: Carcinoma[f]					
Overall rate[a]	1/48 (2%)	6/48 (13%)	12/47 (26%)	3/48 (6%)	8/47 (17%)
Adjusted rate[b]	2.3%	14.4%	30.3%	8.1%	24.4%
Terminal rate[c]	1/34 (3%)	2/28 (7%)	5/21 (24%)	1/23 (4%)	2/13 (15%)
First incidence (days)[d]	737 (T)	676	632	585	416
Poly-3 test[e]	P=0.046	P=0.050	P<0.001	P=0.253	P=0.004
Clitoral Gland: Squamous Cell Papilloma					
Overall rate	0/48 (0%)	0/48 (0%)	1/47 (2%)	0/48 (0%)	3/47 (6%)
Adjusted rate	0%	0%	2.6%	0%	9.3%
Terminal rate	0/34 (0%)	0/28 (0%)	0/21 (0%)	0/23 (0%)	1/13 (8%)
First incidence (days)	-	-	726	-	418
Poly-3 test	P=0.010	-	P=0.475	-	P=0.075
Heart: Malignant Schwannoma[g]					
Overall rate	2/48 (4%)	1/48 (2%)	0/48 (0%)	2/48 (4%)	4/48 (8%)
Adjusted rate	4.6%	2.4%	0%	5.5%	12.3%
Terminal rate	2/34 (6%)	1/28 (4%)	0/21 (0%)	1/23 (4%)	2/13 (15%)
First incidence (days)	737 (T)	737 (T)	-	613	723
Poly-3 test	P=0.047	P=0.515N	P=0.261N	P=0.634	P=0.217
Liver: Hepatocellular Adenoma[h]					
Overall rate	0/48 (0%)	0/48 (0%)	1/48 (2%)	1/48 (2%)	3/48 (6%)
Adjusted rate	0%	0%	2.6%	2.8%	9.3%
Terminal rate	0/34 (0%)	0/28 (0%)	0/21 (0%)	1/23 (4%)	2/13 (15%)
First incidence (days)	-	-	725	737 (T)	709
Poly-3 test	P=0.010	-	P=0.479	P=0.465	P=0.076
Mammary Gland: Fibroadenoma[i]					
Overall rate	16/48 (33%)	18/48 (38%)	24/46 (52%)	22/47 (47%)	31/48 (65%)
Adjusted rate	36.4%	42.2%	59.0%	58.7%	84.2%
Terminal rate	12/34 (35%)	13/28 (46%)	12/21 (57%)	16/23 (70%)	13/13 (100%)
First incidence (days)	656	579	376	501	474
Poly-3 test	P<0.001	P=0.371	P=0.027	P=0.033	P<0.001
Oral Mucosa: Squamous Cell Papilloma[j]					
Overall rate	0/48 (0%)*	2/48 (4%)	1/48 (2%)	2/48 (4%)	4/48 (8%)*
Oral Mucosa: Squamous Cell Carcinoma[j]					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)
Tongue: Squamous Cell Papilloma[j]					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)	1/48 (2%)
Tongue: Squamous Cell Carcinoma[j]					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)
Oral Mucosa or Tongue: Squamous Cell Papilloma or Carcinoma[k]					
Overall rate	0/48 (0%)	2/48 (4%)	1/48 (2%)	3/48 (6%)	5/48 (10%)
Adjusted rate	0%	4.8%	2.6%	8.2%	15.0%
Terminal rate	0/34 (0%)	1/28 (4%)	1/21 (5%)	1/23 (4%)	2/13 (15%)
First incidence (days)	-	519	737 (T)	663	474
Poly-3 test	P=0.004	P=0.231	P=0.479	P=0.092	P=0.014

TABLE 17
Statistical Analysis of Neoplasms in Female Rats
in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, or Sarcoma[l]					
Overall rate	1/48 (2%)	0/48 (0%)	0/48 (0%)	1/48 (2%)	5/48 (10%)
Adjusted rate	2.3%	0%	0%	2.8%	15.4%
Terminal rate	1/34 (3%)	0/28 (0%)	0/21 (0%)	1/23 (4%)	3/13 (23%)
First incidence (days)	737 (T)	-	-	737 (T)	719
Poly-3 test	P=0.001	P=0.509N	P=0.521N	P=0.720	P=0.050
Thyroid Gland: Follicular Cell Adenoma					
Overall rate	0/48 (0%)	0/48 (0%)	1/48 (2%)	0/48 (0%)	2/47 (4%)
Adjusted rate	0%	0%	2.6%	0%	6.3%
Terminal rate	0/34 (0%)	0/28 (0%)	1/21 (5%)	0/23 (0%)	1/13 (8%)
First incidence (days)	-	-	737 (T)	-	724
Poly-3 test	P=0.052	-	P=0.479	-	P=0.177
Thyroid Gland: Follicular Cell Carcinoma[m]					
Overall rate	0/48 (0%)	0/48 (0%)	1/48 (2%)	3/48 (6%)	2/47 (4%)
Adjusted rate	0%	0%	2.6%	8.2%	6.3%
Terminal rate	0/34 (0%)	0/28 (0%)	0/21 (0%)	2/23 (9%)	2/13 (15%)
First incidence (days)	-	-	642	679	737 (T)
Poly-3 test	P=0.031	-	P=0.481	P=0.091	P=0.177
Thyroid Gland: Follicular Cell Adenoma or Carcinoma[n]					
Overall rate	0/48 (0%)	0/48 (0%)	2/48 (4%)	3/48 (6%)	4/47 (9%)
Adjusted rate	0%	0%	5.1%	8.2%	12.5%
Terminal rate	0/34 (0%)	0/28 (0%)	1/21 (5%)	2/23 (9%)	3/13 (23%)
First incidence (days)	-	-	642	679	724
Poly-3 test	P=0.003	-	P=0.216	P=0.091	P=0.031

[a] Number of animals with neoplasm per number of animals examined microscopically.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[c] Observed incidence at the terminal sacrifice.
[d] T indicates terminal sacrifice.
[e] Beneath the 0 mM acrylamide are the p values associated with the trend test. Beneath the treated group incidences are the p values corresponding to pairwise comparisons between the 0 mM acrylamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice. An N indicates a negative trend or decreased tumor incidence.
[f] The historical incidence of clitoral carcinoma in NCTR control female F344/N rats is 5.1% (range 0.0% to 10.4%; Table B3b).
[g] The historical incidence of heart schwannomas in NCTR control female F344/N rats is 0.0% (Table B3f).
[h] The historical incidence of liver hepatocellular adenoma in NCTR control female F344/N rats is 0.3% (range 0.0% to 2.1%; Table B3g).
[i] The historical incidence of mammary gland fibroadenoma in NCTR control female F344/N rats is 35.0% (range 27.1% to 42.6%; Table B3c).
[j] Neoplasm detected at gross necropsy and confirmed by histopathology
[k] The historical incidence of squamous cell carcinoma or papilloma (combined) of the oral cavity in NCTR control female F344/N rats is 0.4% (range 0.0% to 2.1%; Table B3d).
[l] The historical incidence of mesenchymal skin tumors in NCTR control female F344/N rats is 1.0% (range 0.0% to 2.1%; Table B3e).
[m] The historical incidence of thyroid gland follicular cell carcinoma in NCTR control female F344/N rats is 0.0% (Table B3a).
[n] The historical incidence of thyroid gland follicular cell adenoma or carcinoma in NCTR control female F344/N rats is 1.1% (range 0.0% to 2.9%; Table B3a).

Nonneoplastic Findings

Drinking water administration of acrylamide to F334/N rats resulted in degeneration of the retina in the eyes of both sexes. In male rats, the incidence of degeneration was increased in the 0.70 mM group (Table 18), while in female rats the incidence was increased in both the 0.35 and 0.70 mM groups (Table 19). Microscopically, the degenerative changes in the retina were characterized by loss of photoreceptors with corresponding hypocellularity and reduced thickness in other retinal layers.

Acrylamide administration resulted in a dose-related increasing trend in axonal degeneration of the sciatic nerve in both sexes of rats, with the incidence being significant in the 0.70 mM acrylamide treatment groups (Tables 18 and 19). The degeneration was characterized microscopically by dilated axons containing one or two clear vacuoles with small amounts of myelin and/or myelin debris, or one or two foamy macrophages. If three or fewer degenerating axons were observed in a nerve section, the lesions were graded as minimal.

Acrylamide-treated male rats had an increased prevalence of duct ectasia in preputial glands, with the increase being significant in the 0.175, 0.35, and 0.70 mM groups (Table 18). Microscopically this lesion was characterized by dilatation of the main ducts that were usually filled with keratin. Inflammation of varied severity was usually also present. The diagnosis of duct ectasia was recorded as the microscopic correlate to gross observations of preputial gland enlargement in cases where other microscopic lesions that would account for the gross lesion were unapparent. Although no proliferative lesions were observed in these glands, mild hyperplastic changes may have been obscured by the ductal dilatation and inflammation.

Two nonneoplastic lesions (focal hypertrophy and diffuse cytoplasmic vacuolation) involving the adrenal cortex were related to acrylamide treatment in female rats, with the incidence being significantly increased in the 0.70 mM group (Table 19). The focal hypertrophy consisted of distinct focal enlargement of cortical cells in the zona glomerulosa or zona fasiculata. There was no compression of adjacent tissue. The cytoplasm was usually eosinophilic and finely granular and some contained a few lipid vacuoles although the cellular enlargement was not due primarily to lipid accumulation. The cytoplasmic vacuolation consisted of either a focal or diffuse increase in lipid vacuoles in the cytoplasm of cells in the zona fasiculata or zona reticularis.

Female rats administered 0.70 mM acrylamide had an increased prevalence of excessive hematopoietic cell proliferation in the spleen (Table 19). In most of these cases slight enlargement of the spleen had been observed grossly. Microscopic examination revealed that increased hematopoietic activity was the cause of splenic enlargement.

Other dose-related nonneoplastic lesions that showed significant increases in the 0.35 and/or 0.70 mM groups included bone marrow hyperplasia and ovarian atrophy in female rats (Table 19).

TABLE 18
Statistical Analysis of Selected Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Acrylamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Eye					
Retina Degeneration					
Number examined microscopically	44	47	47	46	45
Overall rate[a]	2/44 (5%)	2/47 (4%)	3/47 (6%)	2/46 (4%)	10/45 (22%)
Adjusted rate[b]	5.8%	5.9%	8.0%	5.5%	31.4%
Terminal rate[c]	2/17 (12%)	1/14 (7%)	2/19 (11%)	1/16 (6%)	4/9 (44%)
First incidence (days)[d]	737 (T)	602	666	495	577
Poly-3 test[e]	P<0.001	P=0.688	P=0.539	P=0.673N	P=0.006
Average severity[f]	2.5	3.0	2.0	3.5	2.0
Peripheral Nerve (Sciatic)					
Axon Degeneration					
Number examined microscopically	48	48	48	48	48
Overall rate	5/48 (10%)	7/48 (15%)	7/48 (15%)	11/48 (23%)	23/48 (48%)
Adjusted rate	13.6%	19.7%	18.6%	28.8%	59.8%
Terminal rate	2/17 (12%)	2/14 (14%)	4/19 (21%)	5/16 (31%)	7/9 (78%)
First incidence (days)	557	535	690	662	414
Poly-3 test	P<0.001	P=0.353	P=0.395	P=0.089	P<0.001
Average severity	1.0	1.0	1.0	1.0	1.0
Preputial Gland					
Duct Ectasia					
Number examined microscopically	48	47	48	48	48
Overall rate	4/48 (8%)	6/47 (13%)	11/48 (23%)	14/48 (29%)	10/48 (21%)
Adjusted rate	10.9%	16.9%	28.6%	35.3%	29.4%
Terminal rate	2/17 (12%)	1/14 (7%)	7/19 (37%)	6/16 (38%)	4/9 (44%)
First incidence (days)	555	324	578	578	548
Poly-3 test	P=0.028	P=0.341	P=0.047	P=0.009	P=0.044
Average severity	2.5	2.8	2.6	2.7	2.4

[a] Number of animals with lesion per number of animals examined microscopically.
[b] Poly-3 estimated lesion incidence after adjustment for intercurrent mortality.
[c] Observed incidence at the terminal sacrifice.
[d] T indicates terminal sacrifice.
[e] Beneath the 0 mM acrylamide are the p values associated with the trend test. Beneath the treated group incidences are the p values corresponding to pairwise comparisons between the 0 mM acrylamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice. An N indicates a negative trend or decreased incidence.
[f] Severity was graded as 1, minimal; 2, mild; 3, moderate; and 4, marked.

TABLE 19
Statistical Analysis of Selected Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Acrylamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Adrenal Cortex					
Hypertrophy					
Number examined microscopically	48	48	48	48	48
Overall rate[a]	4/48 (8%)	5/48 (10%)	5/48 (10%)	4/48 (8%)	10/48 (21%)
Adjusted rate[b]	9.3%	12.0%	12.6%	10.9%	28.8%
Terminal rate[c]	4/34 (12%)	1/28 (4%)	1/21 (5%)	3/23 (13%)	2/13 (15%)
First incidence (days)[d]	737 (T)	668	618	523	474
Poly-3 test[e]	P=0.013	P=0.480	P=0.448	P=0.554	P=0.025
Average severity[f]	2.0	2.4	2.6	2.3	2.5
Cytoplasmic Vacuolization					
Number examined microscopically	48	48	48	48	48
Overall rate	2/48 (4%)	5/48 (10%)	5/48 (10%)	5/48 (10%)	9/48 (19%)
Adjusted rate	4.5%	11.7%	12.3%	13.0%	25.3%
Terminal rate	0/34 (0%)	0/28 (0%)	0/21 (0%)	0/23 (0%)	2/13 (15%)
First incidence (days)	512	513	564	428	474
Poly-3 test	P=0.007	P=0.203	P=0.182	P=0.165	P=0.008
Average severity	3.5	2.6	3.2	2.6	2.6
Bone Marrow					
Hyperplasia					
Number examined microscopically	48	48	48	47	48
Overall rate	0/48 (0%)	1/48 (2%)	1/48 (2%)	3/47 (6%)	4/48 (8%)
Adjusted rate	0%	2.4%	2.5%	7.8%	11.3%
Terminal rate	0/34 (0%)	0/28 (0%)	0/21 (0%)	0/23 (0%)	0/13 (0%)
First incidence (days)	-	704	402	257	352
Poly-3 test	P=0.008	P=0.492	P=0.483	P=0.099	P=0.039
Average severity	-	2.0	3.0	2.3	2.3
Eye					
Retina Degeneration					
Number examined microscopically	45	48	47	45	46
Overall rate	14/45 (31%)	16/48 (33%)	16/47 (34%)	21/45 (47%)	23/46 (50%)
Adjusted rate	33.3%	37.0%	39.9%	57.0%	67.3%
Terminal rate	10/34 (29%)	9/28 (32%)	11/21 (52%)	15/23 (65%)	11/13 (85%)
First incidence (days)	677	519	376	404	352
Poly-3 test	P<0.001	P=0.448	P=0.347	P=0.026	P=0.002
Average severity	1.6	1.7	2.2	1.8	2.1
Ovary					
Atrophy					
Number examined microscopically	48	48	48	48	48
Overall rate	38/48 (79%)	41/48 (85%)	43/48 (90%)	44/48 (92%)	43/48 (90%)
Adjusted rate	80.3%	88.0%	90.9%	96.4%	95.2%
Terminal rate	26/34 (77%)	25/28 (89%)	19/21 (91%)	23/23 (100%)	13/13 (100%)
First incidence (days)	463	513	376	257	352
Poly-3 test	P=0.010	P=0.226	P=0.113	P=0.013	P=0.025
Average severity	2.4	2.3	2.5	2.8	2.6
Peripheral Nerve (Sciatic)					
Axon Degeneration					
Number examined microscopically	48	48	48	48	48
Overall rate	4/48 (8%)	3/48 (6%)	1/48 (2%)	4/48 (8%)	19/48 (40%)
Adjusted rate	9.2%	7.2%	2.6%	10.9%	52.5%
Terminal rate	3/34 (9%)	2/28 (7%)	1/21 (5%)	2/23 (9%)	8/13 (62%)
First incidence (days)	691	655	737 (T)	613	416
Poly-3 test	P<0.001	P=0.522N	P=0.213N	P=0.551	P<0.001
Average severity	1.0	1.0	1.0	1.0	1.0

TABLE 19
Statistical Analysis of Selected Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Spleen					
Hematopoietic Cell Proliferation					
Number examined microscopically	48	48	48	48	48
Overall rate	8/48 (17%)	10/48 (21%)	7/48 (15%)	7/48 (15%)	15/48 (31%)
Adjusted rate	18.1%	23.4%	17.2%	18.0%	41.9%
Terminal rate	4/34 (12%)	4/28 (14%)	1/21 (5%)	1/23 (4%)	3/13 (23%)
First incidence (days)	565	522	402	404	416
Poly-3 test	P=0.013	P=0.365	P=0.572N	P=0.610N	P=0.016
Average severity	2.9	2.4	2.7	3.0	3.0

[a] Number of animals with lesion per number of animals examined microscopically.
[b] Poly-3 estimated lesion incidence after adjustment for intercurrent mortality.
[c] Observed incidence at the terminal sacrifice.
[d] T indicates terminal sacrifice.
[e] Beneath the 0 mM acrylamide are the p values associated with the trend test. Beneath the treated group incidences are the p values corresponding to pairwise comparisons between the 0 mM acrylamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice. An N indicates a negative trend or decreased incidence.
[f] Severity was graded as 1, minimal; 2, mild; 3, moderate; and 4, marked.

MICE

2-WEEK STUDY

Mice exposed to 7.03 mM acrylamide in the drinking water did not survive the 14-day treatment period (Table 20). All eight of the mice given 7.03 mM acrylamide in the drinking water were removed from the study after 10 days of exposure due to morbidity. These mice showed a marked weight loss and in the case of two of the mice (one male and one female) hind-leg paralysis. Paralysis was not observed in any other treatments. There were no other significant in-life observations in any of the other treatment groups.

Among mice administered 7.03 mM acrylamide in the drinking water for seven days, males weighed 69% of the control mice and females weighed 74% of the control mice. Male and female mice fed 370 mg acrylamide per kg diet for 14 days weighed 95% and 89% of the control mice, respectively (Table 20). Body weights in the other treatment groups after 14 days of exposure were within 10% of the control mice.

Water and food consumption were relatively constant at each time point and for each dose group (Table 20). Male mice administered 0.14, 0.35, 0.70, 1.41, and 3.52 mM acrylamide in the drinking water (10, 25, 50, 100, or 250 ppm acrylamide) consumed approximately 2.8, 6.8, 13.9, 26.9, and 66.7 mg acrylamide per kg body weight per day, respectively; the comparable values for female mice were 2.9, 7.1, 13.6, 31.0, and 75.8 mg acrylamide per kg body weight per day. The daily intake of acrylamide for mice exposed to 7.03 mM acrylamide could not be determined from the drinking water consumption data but is estimated to be approximately 150 mg acrylamide per kg body weight per day. Male mice fed 7.4, 18.5, 37, 74, 185, and 370 mg acrylamide per kg diet consumed approximately 1.2, 3.1, 6.9, 13.5, 32.8, and 72.8 mg acrylamide per kg body weight per day, respectively; the comparable values for female mice were 1.4, 3.4, 7.0, 14.2, 36.4, and 75.7 mg acrylamide per kg body weight per day.

There were no neoplastic findings in any of the animals. There were no nonneoplastic lesions observed either grossly or microscopically that could be attributed to the administration of acrylamide in the drinking water or acrylamide in the diet.

Exposure Concentration Selection Rationale: Based upon mortality, decreased body weight, and hind-leg paralysis at 7.03 mM acrylamide in drinking water, a high dose of 3.52 mM acrylamide (250 ppm acrylamide) was selected for the subchronic drinking water study, with the remaining doses being 0, 0.14, 0.35, 0.70, and 1.41 mM acrylamide (0, 10, 25, 50, and 100 ppm acrylamide). Based upon the lack of significant adverse effects, a high dose of 370 mg acrylamide per kg diet was selected for the subchronic feeding study, with the remaining doses being 0, 18.5, 37, 74, and 185 mg acrylamide per kg diet. These dose-levels were expected to provide daily doses of acrylamide similar to those obtained by administering 0, 0.14, 0.35, 0.70, 1.41, and 3.52 mM acrylamide in the drinking water.

TABLE 20
Survival, Body Weights, Feed Consumption, and Water Consumption of Mice in the 2-Week Drinking Water and Feed Study of Acrylamide

Treatment	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Mean Feed Consumption[c]		Mean Water Consumption[c]	
		Day 1	Day 7	Day 14		Week 1	Week 2	Week 1	Week 2
Drinking Water									
Male									
0.0 mM	4/4	18.2 ± 0.7	19.9 ± 0.7	17.3 ± 0.3		3.0 (100)	2.5 (100)	4.8 (100)	4.6 (100)
0.14 mM	4/4	17.1 ± 0.5	19.0 ± 0.4	18.0 ± 0.3	104	3.1 (103)	2.5 (100)	5.2 (108)	5.2 (113)
0.35 mM	4/4	18.0 ± 0.2	19.4 ± 0.2	17.0 ± 0.1	98	2.9 (97)	2.5 (100)	5.1 (106)	4.8 (104)
0.70 mM	4/4	18.5 ± 0.4	20.4 ± 0.3	18.7 ± 0.2*	108	3.3 (110)	2.4 (96)	5.8 (121)	5.1 (111)
1.41 mM	4/4	18.6 ± 0.6	19.7 ± 0.6	18.5 ± 0.6	107	3.0 (100)	2.1 (84)	5.0 (104)	5.2 (113)
3.52 mM	4/4	18.8 ± 0.5	18.5 ± 0.2	20.1 ± 0.3*	116	2.6 (87)	4.1 (164)	4.5 (94)	5.8 (126)
7.03 mM	0/4	18.0 ± 0.4	13.6 ± 0.2*			3.6 (120)		9.0[d] (188)	
Female									
0.0 mM	4/4	15.4 ± 0.6	16.1 ± 0.4	14.7 ± 0.5		2.8 (100)	2.1 (100)	5.0 (100)	5.0 (100)
0.14 mM	4/4	15.1 ± 0.6	16.9 ± 0.6	15.2 ± 0.4	103	4.8 (171)	2.2 (105)	4.7 (94)	4.6 (92)
0.35 mM	4/4	14.8 ± 0.6	16.3 ± 0.4	14.5 ± 0.3	99	2.6 (93)	1.8 (86)	4.5 (90)	4.3 (86)
0.70 mM	4/4	15.0 ± 0.4	15.9 ± 0.3	14.2 ± 0.1	96	2.6 (93)	2.1 (100)	4.0 (80)	4.2 (84)
1.41 mM	4/4	15.4 ± 0.7	16.2 ± 0.3	15.6 ± 0.2	106	2.8 (100)	2.7 (129)	4.9 (98)	4.9 (98)
3.52 mM	4/4	14.9 ± 0.8	14.8 ± 0.8	15.2 ± 0.7	104	2.3 (82)	3.1 (148)	4.1 (82)	5.0 (100)
7.03 mM	0/4	14.4 ± 0.6	11.9 ± 0.5*			3.6 (129)		8.9[d] (178)	
Feed									
Male									
0 mg/kg	4/4	16.8 ± 0.9	18.9 ± 0.7	19.4 ± 0.6		3.6 (100)	3.0 (100)	4.2 (100)	4.5 (100)
7.4 mg/kg	4/4	17.8 ± 0.4	19.5 ± 0.4	19.6 ± 0.4	101	3.5 (97)	2.8 (93)	4.2 (100)	4.3 (96)
18.5 mg/kg	4/4	18.6 ± 0.6	20.4 ± 0.3	21.0 ± 0.1	108	3.8 (106)	3.2 (107)	4.8 (114)	4.5 (100)
37 mg/kg	4/4	17.4 ± 0.6	19.8 ± 0.7	20.8 ± 0.5	107	4.0 (111)	3.5 (117)	4.2 (100)	5.5 (122)
74 mg/kg	4/4	16.2 ± 0.4	18.3 ± 0.5	19.7 ± 0.4	101	3.6 (100)	3.3 (110)	4.1 (98)	4.2 (93)
185 mg/kg	4/4	18.1 ± 0.5	19.4 ± 0.5	20.1 ± 0.3	104	3.5 (97)	3.2 (107)	3.7 (88)	4.3 (96)
370 mg/kg	4/4	16.8 ± 0.5	17.2 ± 0.7	18.5 ± 0.9	95	3.7 (103)	3.3 (110)	3.8 (90)	4.2 (93)

TABLE 20
Survival, Body Weights, Feed Consumption, and Water Consumption of Mice in the 2-Week Drinking Water and Feed Study of Acrylamide (continued)

Treatment	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Mean Feed Consumption[c]		Mean Water Consumption[c]	
		Day 0	Day 7	Day 14		Week 1	Week 2	Week 1	Week 2
Feed (continued)									
Female									
0 mg/kg	4/4	14.6 ± 0.5	16.3 ± 0.7	17.7 ± 0.6		3.2 (100)	2.9 (100)	3.5 (100)	4.0 (100)
7.4 mg/kg	4/4	15.0 ± 0.5	16.5 ± 0.4	16.3 ± 0.5	92	3.2 (100)	2.8 (97)	4.1 (117)	4.6 (115)
18.5 mg/kg	4/4	14.1 ± 0.6	16.9 ± 0.6	16.2 ± 0.5	91	3.3 (103)	2.8 (97)	4.0 (114)	4.4 (110)
37 mg/kg	4/4	14.3 ± 0.4	15.7 ± 0.2	16.1 ± 0.1	91	3.2 (100)	2.8 (97)	4.3 (123)	4.2 (105)
74 mg/kg	4/4	13.7 ± 0.2	15.6 ± 0.3	16.4 ± 0.2	93	3.2 (100)	2.9 (100)	4.1 (117)	4.0 (100)
185 mg/kg	4/4	14.0 ± 0.6	15.8 ± 0.4	16.8 ± 0.8	95	3.4 (106)	3.0 (103)	3.7 (106)	4.5 (113)
370 mg/kg	4/4	14.3 ± 0.5	14.6 ± 0.7	15.8 ± 0.8	89	3.2 (100)	3.0 (103)	3.7 (106)	3.9 (98)

[a] Number of animals surviving at 14 days/number initially in group.
[b] Weights are given as mean ± standard error. An asterisk (*) denotes significant difference ($p < 0.05$) from control.
[c] Feed and water consumption are expressed as grams per animal per day and were measured on a per cage basis. Values in parentheses indicate the percentage of controls. Statistical analyses were not conducted on feed and water consumption because there was only one cage per treatment group.
[d] Value not used for calculating daily acrylamide exposure.

3-MONTH STUDY

Two animals died and one was accidentally killed before the end of the experiment: one male mouse fed 185 mg acrylamide per kg diet died on day 20, one male mouse fed 370 mg acrylamide per kg diet died on day 61, and one male mouse fed 18.5 mg acrylamide per kg diet was accidentally killed on day 18 (Table 21). Hind-limb paralysis was observed in all mice administered 3.52 mM acrylamide in the drinking water (Table 22). In female mice the hind-limb paralysis became evident between days 39 and 57 of the experiment; in males the condition became apparent between days 53 and 75. Hind-limb paralysis occurred in all mice administered 370 mg acrylamide per kg diet (Table 23). This was observed after 52 to 54 days of treatment.

Acrylamide in the drinking water caused significant dose-related effects on body weight in both male and female B6C3F1 mice (Figure 10 and Table 21). At the end of the 13-week period, the male mice administered 1.41 and 3.52 mM acrylamide weighed 91% and 86% of the control group; female mice administered 3.52 mM acrylamide weighed 94% of the control group. Acrylamide in the diet caused significant dose-related effects on body weight in both male and female mice (Figure 11 and Table 21). Treatment with 370 mg acrylamide per kg diet resulted in significant decreases in body weight gain in both sexes. At the end of the 13-week period, mice treated with 370 mg acrylamide per kg diet weighed 81% (females) and 87% (males) of their respective control groups.

Male mice administered 0.0, 0.14, 0.35, 0.70, 1.41, and 3.52 mM acrylamide in the drinking water (0, 10, 25, 50, 100, or 250 ppm acrylamide) consumed approximately 6.3, 7.6, 6.8, 6.4, 7.6, and 6.2 ml drinking water per day, which was equivalent to 0.0, 3.2, 6.9, 13.3, 32.8, and 70.0 mg acrylamide per kg body weight per day; the comparable values for female mice were 5.6, 6.6, 5.9, 6.1, 5.9, and 5.7 ml drinking water per day, which was equivalent to 0.0, 3.5, 7.8, 16.4, 31.4, and 83.1 mg acrylamide per kg body weight per day. Male mice fed 0.0, 18.5, 37, 74, 185, and 370 mg acrylamide per kg diet consumed approximately 6.1, 8.3, 7.1, 6.6, 6.8 and 5.5 ml drinking water per day; the comparable values for female mice were 5.1, 5.0, 5.5, 5.0, 5.8, and 4.7 ml drinking water per day.

Male mice administered 0.0, 0.14, 0.35, 0.70, 1.41, and 3.52 mM acrylamide in the drinking water (0, 10, 25, 50, 100, and 250 ppm acrylamide) consumed approximately 3.6, 3.5, 3.3, 3.7, 3.3, and 3.1 g feed per day; the comparable values for female mice were 3.9, 4.1, 4.0, 3.9, 3.7, and 3.6 g feed per day. Male mice fed 0.0, 18.5, 37, 74, 185, and 370 mg acrylamide per kg diet consumed approximately 3.8, 4.1, 3.9, 3.5, 3.7, and 3.2 g feed per day, which was equivalent to 0.0, 3.3, 6.6, 12.0, 32.1, and 59.4 mg acrylamide per kg body weight per day; the comparable values for female mice were 3.5, 3.7, 3.8, 3.4, 3.4, and 2.8 g feed per day, which was equivalent to 0.0, 3.7, 7.5, 13.9, 35.1, and 64.0 mg acrylamide per kg body weight per day.

Necropsy body weights were decreased in male mice administered 0.14, 0.70, 1.41 and 3.52 mM acrylamide and female mice administered 3.52 mM acrylamide in the drinking water for 13 weeks (Table E7). The liver weights and liver weight to body weight ratios were increased in male mice administered 1.41 mM acrylamide, and the brain weights were decreased in male and female mice administered 3.52 mM acrylamide. Necropsy body weights, liver weights, and brain weights were decreased in male and female mice fed 370 mg acrylamide per kg diet for 13 weeks (Table E8).

The only gross observation in the mice given acrylamide in the drinking water that was considered to be treatment related was marked dilatation of the urinary bladder in the 3.52 mM groups. This change was evident in all eight males and in five of eight females. In the mice administered acrylamide in the diet, all seven males and seven of eight females in the 370 mg/kg group had a dilated urinary bladder. All of these animals also had a clinical observation of partial paralysis of the rear legs.

In both drinking water and dietary routes of administration, treatment-related changes were observed in the following target tissues: sciatic nerve, spinal cord, skeletal muscle of the hind-limb, testes, and ovaries. Target tissues were examined microscopically in progressively lower dose groups until a no-observed-adverse-effect level was reached. In mice administered acrylamide in the drinking water, the most significant treatment-related change was a radiculoneuropathy involving the sciatic nerve and lumbar spinal cord (lateral funiculus). The lesion was observed in the sciatic nerve in all male and female mice administered 3.52 mM acrylamide in the drinking water and in the spinal cord of all male and seven of eight female mice treated with 3.52 mM acrylamide (Table 22).

The radiculoneuropathy was characterized by nerve fiber degeneration with dilatation and vacuolization of myelin sheaths along with swollen and shrunken axons,

TABLE 21
Survival and Body Weights of Mice in the 3-Month Drinking Water and Feed Studies of Acrylamide

Treatment	Survival[a]	Mean Body Weight (g)[b]		Final weight Relative to Controls (%)
		Week 0	Week 14[c]	
Drinking Water				
Male				
0.0 mM	8/8	18.4 ± 0.3	28.5 ± 0.3	
0.14 mM	8/8	17.8 ± 0.3	25.3 ± 0.3	89
0.35mM	8/8	18.3 ± 0.3	27.1 ± 0.3	95
0.70 mM	8/8	16.8 ± 0.3	26.2 ± 0.3	92
1.41 mM	8/8	17.0 ± 0.3	25.8 ± 0.3	91
3.52 mM	8/8	17.9 ± 0.3	24.5 ± 0.3	86
Female				
0.0 mM	8/8	14.0 ± 0.2	20.9 ± 0.2	
0.14 mM	8/8	13.9 ± 0.2	21.8 ± 0.2	104
0.35 mM	8/8	13.7 ± 0.2	21.7 ± 0.2	104
0.70 mM	8/8	14.4 ± 0.2	20.8 ± 0.2	100
1.41 mM	8/8	15.2 ± 0.2	21.4 ± 0.2	102
3.52 mM	8/8	14.4 ± 0.2	19.7 ± 0.2	94
Feed				
Male				
0 mg/kg	8/8	15.4 ± 0.4	26.1 ± 0.4	
18.5 mg/kg	7/8[d]	15.6 ± 0.4	28.1 ± 0.4	108
37 mg/kg	8/8	15.8 ± 0.4	26.5 ± 0.4	102
74 mg/kg	8/8	15.5 ± 0.4	26.7 ± 0.4	102
185 mg/kg	7/8	15.4 ± 0.4	26.5 ± 0.4	102
370 mg/kg	7/8	14.8 ± 0.4	22.7 ± 0.4	87
Female				
0 mg/kg	8/8	12.9 ± 0.2	22.2 ± 0.2	
18.5 mg/kg	8/8	13.1 ± 0.2	22.9 ± 0.2	103
37 mg/kg	8/8	13.0 ± 0.2	21.5 ± 0.2	97
74 mg/kg	8/8	13.1 ± 0.2	21.0 ± 0.2	95
185 mg/kg	8/8	13.2 ± 0.2	21.8 ± 0.2	98
370 mg/kg	8/8	13.3 ± 0.2	17.9 ± 0.2	81

[a] Number of animal surviving until study termination/number of animals initially in group.
[b] Weights are given as LS means ± standard error of the mean.
[c] Final body weights from the feed study were measured at week 15.
[d] One male mouse from the 18.5 mg/kg group was accidently killed on day 18.

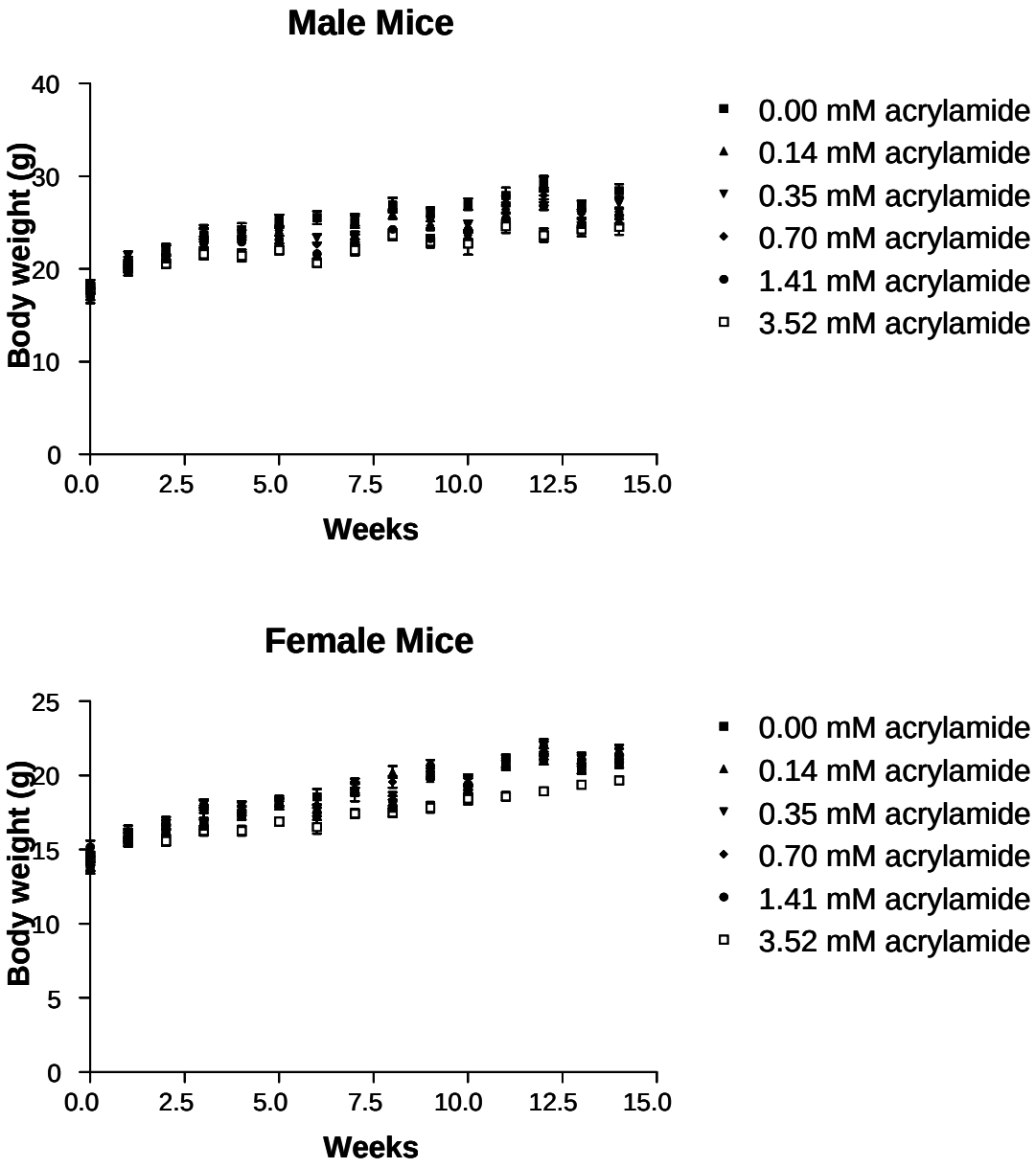


FIGURE 10
Growth Curves for Male and Female Mice
in the 3-Month Drinking Water Study of Acrylamide

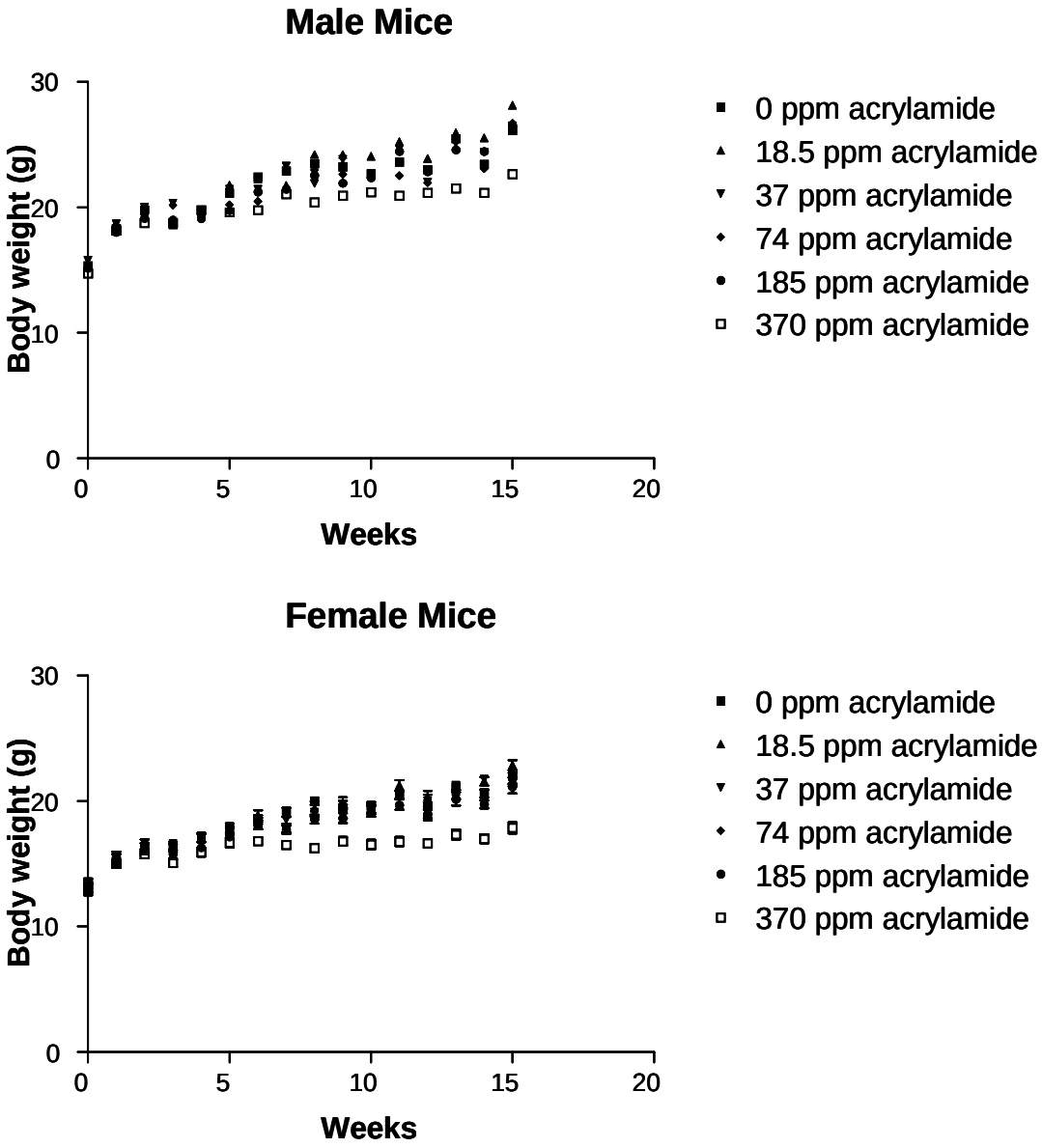


FIGURE 11
Growth Curves of Male and Female Mice in the 3-Month Feed Study of Acrylamide

TABLE 22
Incidence of Observations and Nonneoplastic Lesions in Mice in the 3-Month Drinking Water Study of Acrylamide[a]

	0 mM	0.14 mM	0.35 mM	0.70 mM	1.41 mM	3.52 mM
Males						
Animals initially in study	8	8	8	8	8	8
Hind-leg						
Paralysis	0/8	0/8	0/8	0/8	0/8	8/8
Peripheral nerve						
Axon degeneration	0/8	-[b]	-	0/8	0/8	8/8 (1.0)
Spinal cord						
Lumbar axon degeneration	0/8	-	-	-	0/8	8/8 (1.0)
Skeletal muscle						
Atrophy	0/8	-	-	0/8	0/8	3/8 (1.3)
Urinary bladder						
Dilatation	0/8	-	-	-	-	8/8 (3.9)
Testes						
Germinal epithelium degeneration	0/8	-	-	-	0/8	6/8 (1.3)
Females						
Animals initially in study	8	8	8	8	8	8
Hind-leg						
Paralysis	0/8	0/8	0/8	0/8	0/8	8/8
Peripheral nerve						
Axon degeneration	0/8	-	-	0/8	0/8	8/8 (1.4)
Spinal cord						
Lumbar axon degeneration	0/8	-	-	-	0/8	7/8 (1.0)
Skeletal muscle						
Atrophy	0/8	-	-	0/8	0/8	5/8 (1.2)
Urinary bladder						
Dilatation	0/8	-	-	-	-	4/8 (2.8)
Ovary						
Anestrus	0/8	-	-	-	0/8	6/8

[a] Data are reported as the number of lesions per number of mice examined microscopically. The average severity is given in parentheses. severity was scored as: 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked.
[b] Not examined.

with a severity of minimal to mild. The neuronal degenerative changes in the mice administered 3.52 mM acrylamide were accompanied by minimal to mild muscle atrophy involving the rear legs in three of eight male mice and five of eight female mice and mild to marked luminal dilatation of the urinary bladder of all eight male mice and four of eight female mice (Table 22).

In mice administered acrylamide in the diet, the most significant treatment-related change was a minimal to mild radiculoneuropathy (degenerative lesion) involving the sciatic nerve. The lesion was observed in five of seven male mice and all seven female mice administered 370 mg acrylamide per kg diet and in one of eight female mice administered 185 mg acrylamide per kg diet (Table 23). The histopathologic characteristics of this lesion were very similar to those in observed in mice given acrylamide in the drinking water. Degenerative changes of the lumbar spinal cord, of minimal severity, were also present in two of seven male mice fed 370 mg acrylamide per kg diet. The neuronal degenerative changes in the mice administered 370 mg acrylamide per kg diet were accompanied by minimal to mild muscle atrophy involving the rear legs in seven of seven male mice and seven of eight female mice and moderate to marked luminal dilatation of the urinary bladder in seven of seven male mice and seven of eight female mice.

Examination of the female reproductive organs indicated that six of eight mice given 3.52 mM acrylamide in the drinking water were in anestrus (Table 22). The change was characterized by the absence of corpora lutea in various stages of development or in regression from previous ovulations. Anestrus was observed in all eight female mice given 370 mg acrylamide per kg diet (Table 23). In male mice, minimal to mild depletion of the testicular germinal cell epithelium occurred in six of eight mice treated with 3.52 mM acrylamide (Table 22). In male mice fed 370 mg acrylamide per kg diet, mild depletion of the testicular germinal cell epithelium was observed in all seven mice and moderate hypospermia of the epididymis occurred in three of seven mice (Table 23).

TABLE 23
Incidence of Observations and Nonneoplastic Lesions in Mice in the 3-Month Feed Study of Acrylamide[a]

	0 mg/kg	18.5 mg/kg	37 mg/kg	74 mg/kg	185 mg/kg	370 mg/kg
Males						
Animals initially in study	8	8	8	8	8	8
Hind-leg						
Paralysis	0/8	0/8	0/8	0/8	0/8	8/8
Peripheral nerve						
Axon degeneration	0/8	-[b]	-	-	0/8	5/7 (1.2)
Spinal cord						
Lumbar axon degeneration	0/8	-	-	-	0/8	2/7 (1.0)
Skeletal muscle						
Atrophy	0/8	-	-	-	0/8	7/7 (1.3)
Urinary bladder						
Dilatation	0/8	-	-	-	0/1	7/7 (4.0)
Testes						
Germinal epithelium degeneration	0/8	-	-	-	0/8	7/7 (2.1)
Epididymis						
Hypospermia	0/8	-	-	-	0/1	3/7 (2.7)
Females						
Animals initially in study	8	8	8	8	8	8
Hind-leg						
Paralysis	0/8	0/8	0/8	0/8	0/8	8/8
Peripheral nerve						
Axon degeneration	0/8	-	-	-	1/8 (1.0)	7/7 (1.9)
Spinal cord						
Lumbar axon degeneration	0/8	-	-	-	0/8	0/8
Skeletal muscle						
Atrophy	0/8	-	-	-	0/8	7/8 (2.0)
Urinary bladder						
Dilatation	0/8	-	-	-	-	7/8 (3.3)
Ovary						
Anestrus	0/8	-	-	-	0/8	8/8

[a] Data are reported as the number of lesions per number of mice examined microscopically. The average severity is given in parentheses. Severity was scored as: 1 = minimal; 2 = mild; 3 = moderate; and 4 = marked.
[b] Not examined.

Exposure Concentration Selection Rationale: Mice receiving 3.52 mM acrylamide in the drinking water for 13 weeks had decreased body weights, hind-limb paralysis, urinary bladder dilatation, and radiculo-neuropathy, which was typically accompanied by skeletal muscle atrophy. Because a decrease of body weight gain of approximately 10% was noted in the 1.41 mM acrylamide dose, a high dose of 0.70 mM acrylamide in drinking water was selected for the 2-year study.

As noted in the Study Rationale, one objective of this study was to compare the induction of tumors by acrylamide with that of its metabolite glycidamide, as a function of dose, in B6C3F1 mice. In the range finding and subchronic studies in B6C3F1 mice, acrylamide gave similar responses when administered in the diet and in the drinking water. As noted earlier, glycidamide rapidly decomposes when mixed in the diet; thus, only drinking water exposures were used in the 2-year study phase of the experiment to allow a direct comparison between the responses induced by acrylamide with those induced by glycidamide.

2-YEAR STUDY

Survival and Cause of Death

Acrylamide in the drinking water caused a dose-related decreasing trend in survival in male and female B6C3F1 mice (Table 24 and Figure 12). Compared to control mice, male B6C3F1 mice administered 0.70 mM acrylamide and female B6C3F1 mice administered 0.35 and 0.70 mM acrylamide had decreased survival. The cause for more than 85% of the early removals or deaths of these mice was neoplasms, including malignant lymphoma, leukemia (females only), mammary gland adenoacanthoma or adenocarcinoma (females only), harderian gland adenoma, and various types of sarcoma.

TABLE 24
Survival and Disposition of Mice in the 2-Year Drinking Water Study of Acrylamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Male					
Animals initially in study	48	48	48	48	48
Moribund	3	5	8	7	12
Natural deaths	6	4	3	3	8
Animals surviving to study termination[a]	39	39	37	38	28
Percent probability of survival at end of study[b]	81	81	77	79	58
Mean survival (weeks)[c]	91.7	97.3	99.3	97.9	94.0
Survival analysis[d]	P = 0.009	P = 0.929	P = 0.708	P = 0.879	P = 0.026
Female					
Animals initially in study	48	48	48	48	48
Accidental death[a]	0	0	2	0	0
Moribund	6	7	10	20	22
Natural deaths	3	5	0	3	11
Animals surviving to study termination[a]	39	36	36	25	15
Percent probability of survival at end of study[b]	81	75	78	52	31
Mean survival (weeks)[c]	102.7	99.5	102.0	92.4	91.7
Survival analysis[d]	P < 0.001	P = 0.448	P = 0.719	P = 0.002	P <0.001

[a] Censored from the survival analyses.
[b] Kaplan-Meier survival estimates.
[c] Mean of all deaths (censored and uncensored).
[d] The result of the life table trend test (Tarone, 1975) is in the 0.0 μM acrylamide column, and the results of the life table pairwise comparisons (Cox, 1972) with the 0.0 μM acrylamide are in the treatment group columns.

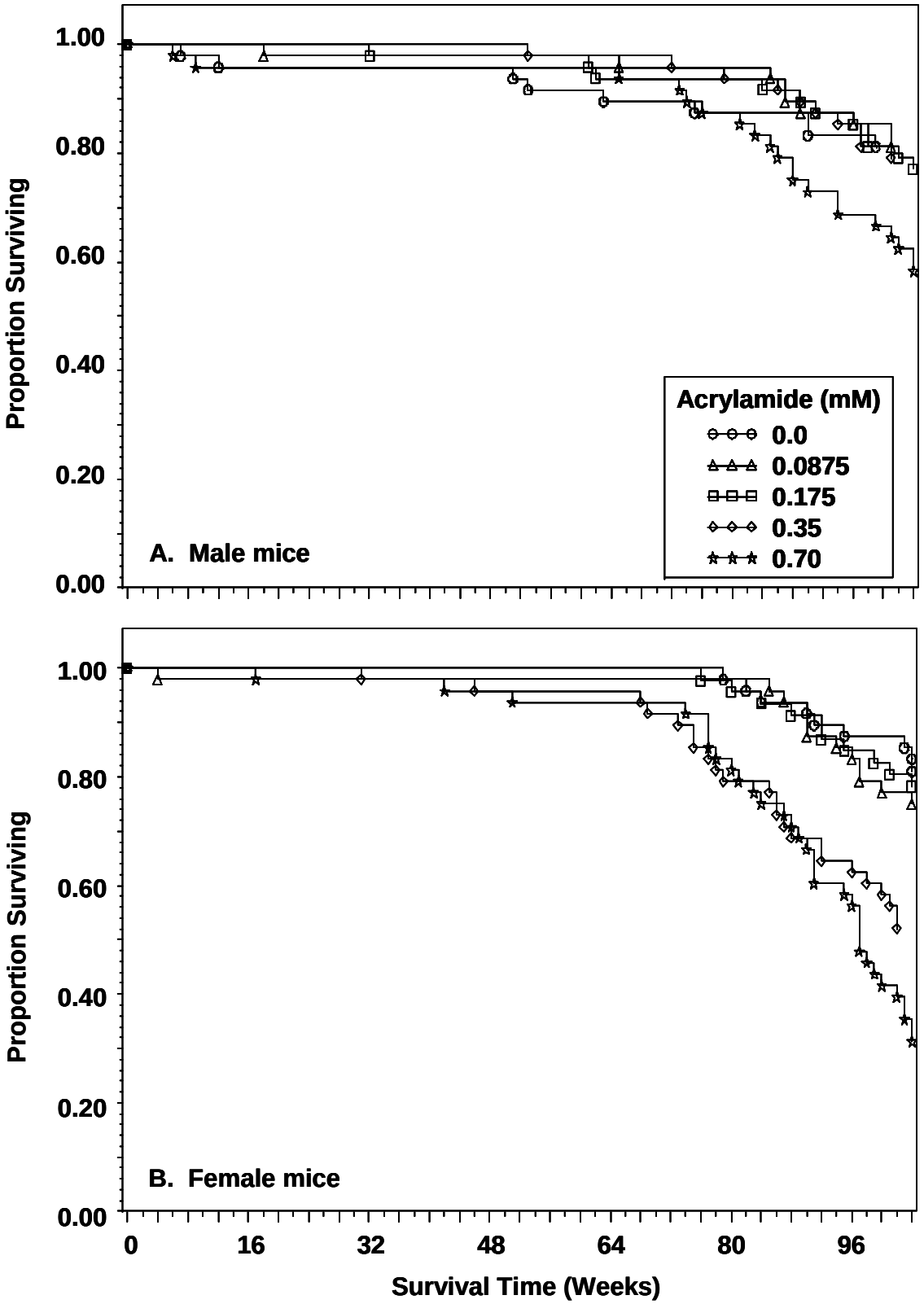


FIGURE 12
Kaplan-Meier Survival Curves for Male and Female Mice Administered Acrylamide in Drinking Water for 2 Years

Body Weights and Feed and Water Consumption

Administering acrylamide in the drinking water to male and female B6C3F1 mice caused only sporadic statistically significant changes in body weights, with the magnitude of the change never exceeding 6% of the mean control body weight at the same time point (Figure 13 and Tables 25 and 26).

Acrylamide in the drinking water caused sporadic dose-related increasing trends in food consumption in male B6C3F1 mice (Table G3), with the food consumption in the 0.70 mM acrylamide group being significantly increased compared to the control group at weeks 88 and 96. In female B6C3F1 mice, acrylamide in the drinking water caused dose-related increasing trends in food consumption beginning at week 84 (Table G4), with the food consumption in the 0.70 mM acrylamide group being significantly increased compared to the control group beginning at week 92.

Acrylamide in the drinking water caused sporadic dose-related increasing trends in water consumption in male B6C3F1 mice (Table 27). In female B6C3F1 mice, there was a dose-related increasing trend in water consumption beginning at week 76 (Table 28). Water consumption in the 0.70 mM acrylamide group of female B6C3F1 mice was significantly increased compared to the control group at weeks 76, 80, and 92 to 104 (Table 28).

The mean acrylamide exposure for the B6C3F1 mice, calculated at 4 week intervals, is presented in Figure 14 and Tables 27 and 28. The mean amount of acrylamide consumed by male B6C3F1 mice for the entire 2 year experiment was 1.04, 2.20, 4.11, and 8.93 mg acrylamide per kg body weight per day for the 0.0875, 0.175, 0.35, and 0.70 mM acrylamide dose groups (6.25, 12.5, 25, and 50 ppm acrylamide), respectively. The corresponding values for female B6C3F1 mice were 1.10, 2.23, 4.65, and 9.96 mg acrylamide per kg body weight per day.

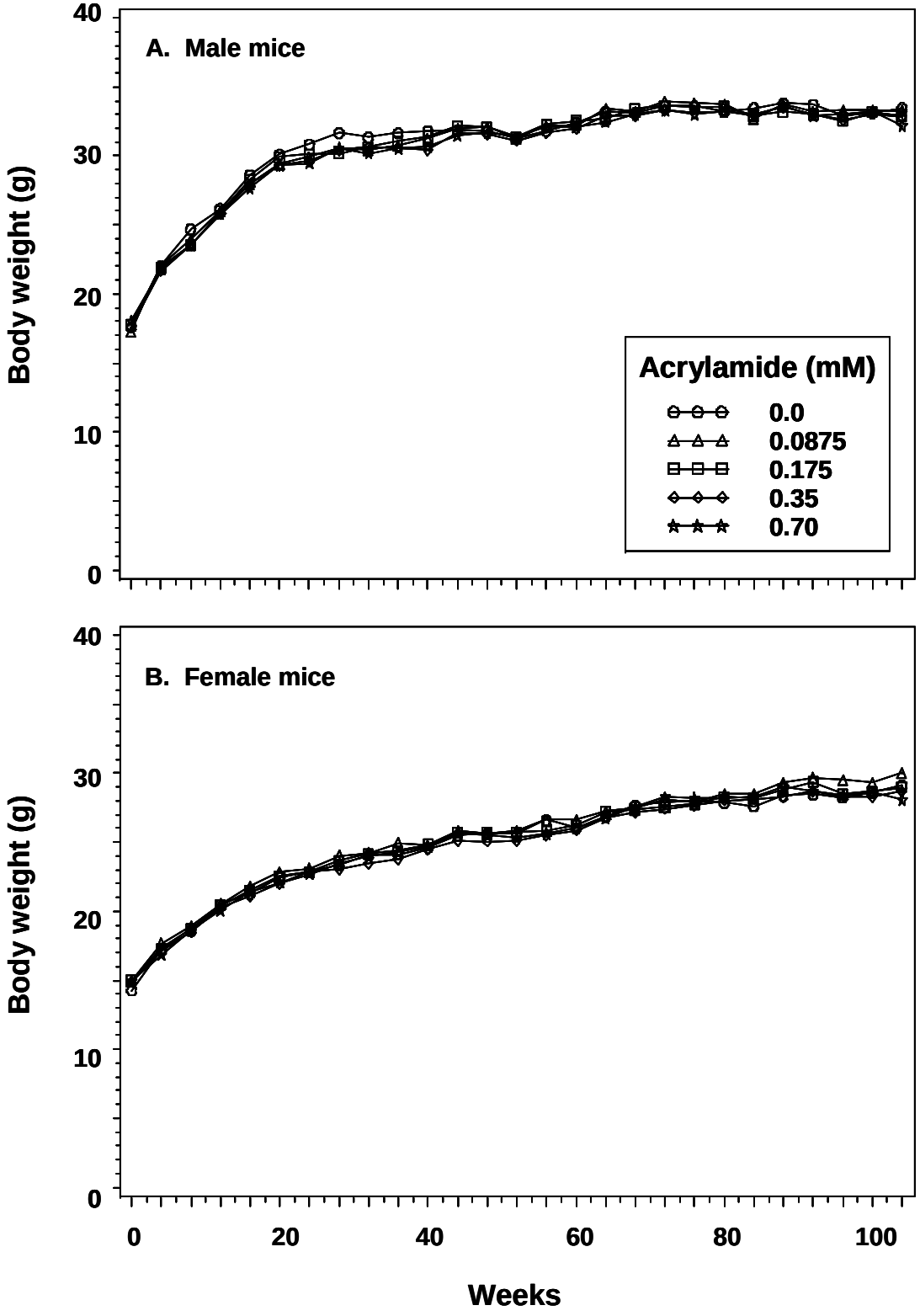


FIGURE 13
Growth Curves for Male and Female Mice
Administered Acrylamide in Drinking Water for 2 Years

TABLE 25
Mean Body Weights[a] and Survival of Male Mice in the 2-Year Drinking Water Study of Acrylamide

Weeks on Study	0 mM		0.0875 mM			0.175 mM			0.35 mM			0.70 mM		
	Mean Wt. (g)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors
4	22.0	48	22.0	100.1	48	21.9	99.6	48	21.7	98.4	48	21.8	98.9	48
8	24.7*	47	24.0	97.1	48	23.6*	95.5	48	23.6*	95.5	48	23.6*	95.9	47
12	26.2	47	26.0	99.4	48	26.0	99.3	48	25.8	98.7	48	25.8	98.6	46
16	28.6*	46	27.9	97.9	48	28.3	99.1	48	28.0	98.0	48	27.7	97.0	46
20	30.1	46	29.4	97.8	47	30.0	99.7	48	29.3	97.6	48	29.3	97.6	46
24	30.8*	46	29.9*	97.2	47	30.1	97.8	48	29.7*	96.3	48	29.5*	95.7	46
28	31.6	46	30.6*	96.6	47	30.2*	95.4	48	30.5*	96.3	48	30.6*	96.7	46
32	31.3*	46	30.5	97.3	47	30.7	97.9	48	30.7	97.9	48	30.2*	96.4	46
36	31.6*	46	30.8	97.3	47	30.9	97.8	47	30.6*	96.7	48	30.5*	96.5	46
40	31.8*	46	31.3	98.3	47	31.3	98.5	47	30.5*	95.9	48	30.6*	96.4	45
44	31.9	46	32.0	100.3	47	32.1	100.7	47	31.7	99.5	48	31.5	98.8	46
48	31.8	46	32.1	101.1	47	32.0	100.8	47	31.5	99.3	48	31.7	99.7	46
52	31.3	45	31.3	99.9	47	31.3	100.0	47	31.1	99.4	48	31.4	100.2	46
56	31.8	44	32.2	101.0	47	32.2	101.2	47	31.6	99.3	47	32.1	100.8	46
60	32.4	44	32.2	99.4	47	32.5	100.3	47	32.0	98.7	47	32.1	98.9	46
64	33.1	43	33.4	101.1	47	32.9	99.6	45	32.9	99.6	47	32.4	98.2	46
68	33.0	43	33.3	100.9	46	33.5	101.7	45	32.9	99.7	47	33.0	100.1	45
72	33.5	43	33.9	101.2	46	33.7	100.5	45	33.3	99.3	47	33.4	99.4	45
76	33.5	42	33.9	101.1	46	33.6	100.4	45	33.1	98.8	46	33.0	98.5	43
80	33.1	42	33.7	101.9	46	33.6	101.6	45	33.2	100.2	45	33.1	100.0	42
84	33.4	42	32.8	98.0	46	33.0	98.7	45	33.0	98.7	45	32.5	97.3	40
88	33.8	42	33.9	100.3	43	33.3	98.6	44	33.5	99.0	44	33.4	99.0	38
92	33.8*	40	33.3	98.5	42	33.1	97.9	42	33.0	97.7	42	32.7	96.9	35
96	32.9	40	33.4	101.3	42	32.6	98.9	42	32.5	98.7	41	32.8	99.6	33
100	33.1	39	33.5	101.2	41	33.0	99.7	39	32.8	99.0	39	33.0	99.7	32
104	33.5*	39	33.5	100.0	39	32.8	98.0	38	32.7	97.8	38	32.4	96.7	30
Mean for weeks														
4-104	31.3		31.2			31.1			30.8			30.8		

[a] An * in the 0.0 mM acrylamide column indicates a significant dose-related trend ($p < 0.05$); an * in a treatment column indicates a significant ($p < 0.05$) pairwise comparison of the dose group to the 0.0 mM acrylamide group as determined by Dunnett's test.

TABLE 26
Mean Body Weights[a] and Survival of Female Mice in the 2-Year Drinking Water Study of Acrylamide

Weeks on Study	0 mM		0.0875 mM			0.175 mM			0.35 mM			0.70 mM		
	Mean Wt. (g)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors
4	17.2*	48	17.7	102.6	48	17.3	100.5	48	17.1	99.3	48	16.8	98.0	48
8	18.6	48	18.9	101.7	47	18.7	100.9	48	18.5	99.8	48	18.7	100.4	48
12	20.5	48	20.5	100.1	47	20.4	99.6	48	20.3	99.0	48	20.1	98.0	48
16	21.3	48	21.8	102.3	47	21.5	101.0	48	21.1	99.0	48	21.4	100.6	48
20	22.4*	48	22.8	101.7	47	22.6	100.5	48	22.0	98.2	48	22.1	98.3	47
24	22.8	48	23.1	101.0	47	22.8	99.8	48	22.9	100.2	48	22.7	99.3	47
28	23.4	48	24.0	102.7	47	23.7	101.3	48	23.1	98.7	48	23.5	100.4	47
32	24.1	48	24.2	100.7	47	24.1	100.4	48	23.5	97.6	47	23.9	99.5	47
36	24.1	48	24.9	103.3	47	24.4	101.1	48	23.8	98.6	47	24.3	100.6	47
40	24.6	48	24.8	100.6	47	24.9	101.0	48	24.5	99.4	47	24.7	100.2	47
44	25.5	48	25.8	101.2	47	25.7	100.9	48	25.1	98.3	47	25.6	100.4	46
48	25.6	48	25.6	100.1	47	25.7	100.2	48	25.1	98.1	46	25.5	99.5	46
52	25.6	48	25.8	100.7	47	25.7	100.2	47	25.2	98.3	46	25.3	98.9	45
56	26.6*	48	26.6	99.9	47	25.8	96.9	47	25.6*	96.1	46	25.6*	96.2	45
60	26.1	48	26.7	102.3	47	26.2	100.5	47	25.9	99.5	46	26.0	99.9	45
64	26.9	48	27.3	101.5	47	27.2	101.2	47	26.9	100.0	46	26.8	99.6	45
68	27.6	48	27.5	99.5	47	27.4	99.0	46	27.2	98.4	46	27.5	99.6	45
72	27.9	48	28.1	100.5	47	27.6	98.9	46	27.6	98.9	44	28.3	101.3	45
76	28.0	48	27.9	99.9	47	27.8	99.3	46	27.8	99.4	41	28.1	100.6	44
80	27.9	47	28.5	102.1	47	28.2	101.2	45	28.4	101.8	38	28.2	101.3	40
84	27.9	46	28.5	102.1	47	28.0	100.3	44	28.4	101.8	38	28.4	101.6	37
88	28.7	45	29.3	102.2	45	28.7	100.0	43	28.8	100.3	34	29.1	101.6	35
92	28.7	43	29.9	104.2	42	29.3	102.0	42	28.9	100.8	33	28.9	100.6	29
96	28.4	42	29.9	105.1	41	28.6	100.7	39	28.8	101.1	31	28.7	100.8	28
100	28.8	42	29.9	103.8	38	28.9	100.3	38	28.8	100.1	29	28.6	99.6	21
104	29.3	41	30.6	104.8	37	29.2	99.7	37	29.7	101.4	25	28.9	98.6	17
Mean for weeks														
4-104	25.3		25.8			25.4			25.2			25.3		

[a] An * in the 0.0 mM acrylamide column indicates a significant dose-related trend ($p < 0.05$); an * in the treatment column indicates a significant ($p < 0.05$) pairwise comparison of the dose group to the 0.0 mM acrylamide group as determined by Dunnett's test.

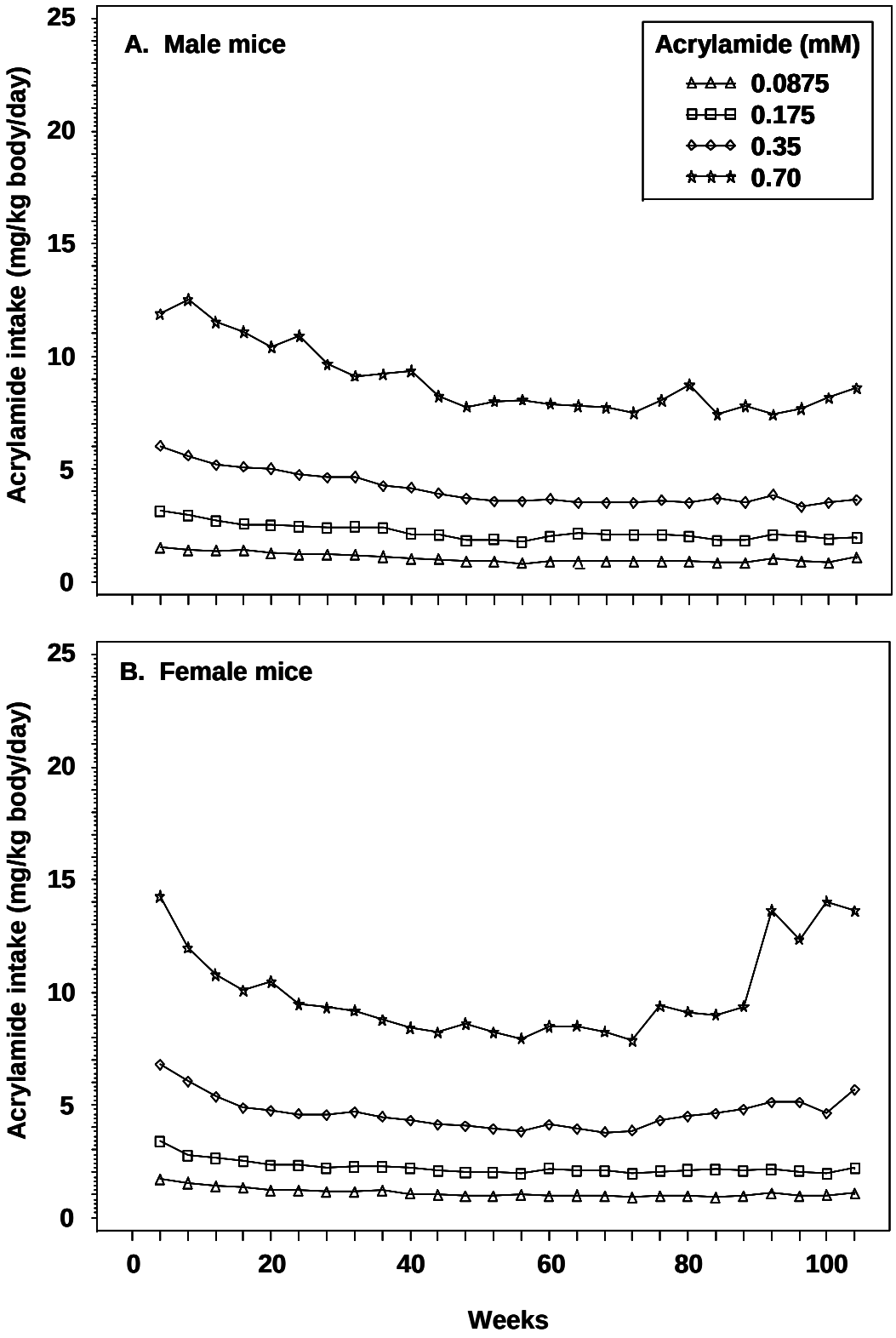


FIGURE 14
Acrylamide Intake in Male and Female Mice Administered Acrylamide in Drinking Water for 2 Years

TABLE 27
Water and Acrylamide Consumption by Male Mice in the 2-Year Drinking Water Study of Acrylamide

Week[a]	0 mM		0.0875 mM			0.175 mM			0.35 mM			0.70 mM		
	Water (g/day)	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose[b]	Water (g/day)	Body Weight (g)	Dose	Water (g/day)	Body Weight (g)	Dose	Water (g/day)	Body Weight (g)	Dose
4	4.9	22.0	4.9	22.0	1.50	5.2	21.9	3.14	5.0	21.7	6.02	5.1	21.8	11.88
8	5.1	24.7	5.2	24.0	1.40	5.5	23.6	2.96	5.1	23.6	5.58	5.4	23.6	12.53
12	5.3	26.2	5.6	26.0	1.37	5.5	26.0	2.69	5.2	25.8	5.18	5.7	25.8	11.54
16	5.7	28.6	6.0	27.9	1.38	5.6	28.3	2.55	5.6	28.0	5.09	5.9	27.7	11.10
20	6.1	30.1	6.0	29.4	1.29	5.9	30.0	2.51	5.8	29.3	5.02	5.8	29.3	10.41
24	5.8	30.8	5.8	29.9	1.20	5.9	30.1	2.43	5.6	29.7	4.73	6.1	29.5	10.90
28	5.7	31.6	5.9	30.6	1.21	5.8	30.2	2.37	5.6	30.5	4.63	5.8	30.6	9.64
32	5.6	31.3	5.7	30.5	1.18	5.9	30.7	2.42	5.7	30.7	4.65	5.5	30.2	9.13
36	5.4	31.6	5.4	30.8	1.11	5.9	30.9	2.40	5.2	30.6	4.25	5.7	30.5	9.20
40	5.2*	31.8	5.1	31.3	1.00	5.4	31.3	2.12	5.1	30.5	4.15	6.0	30.6	9.36
44	4.9	31.9	5.0	32.0	0.99	5.3	32.1	2.06	4.9	31.7	3.91	5.2	31.5	8.22
48	4.8	31.8	4.7	32.1	0.92	4.7	32.0	1.82	4.7	31.5	3.67	5.1	31.7	7.77
52	4.6*	31.3	4.5	31.3	0.90	4.7	31.3	1.87	4.5	31.1	3.59	5.3	31.4	8.01
56	4.5*	31.8	4.2	32.2	0.81	4.6	32.2	1.77	4.5	31.6	3.59	5.3	32.1	8.07
60	4.7	32.4	4.7	32.2	0.92	5.2	32.5	2.02	4.7	32.0	3.66	5.2	32.1	7.88
64	4.9	33.1	4.8	33.4	0.90	5.5	32.9	2.13	4.6	32.9	3.49	5.5	32.4	7.79
68	5.2	33.0	4.7	33.3	0.88	5.4	33.5	2.06	4.6	32.9	3.52	5.2	33.0	7.71
72	4.7	33.5	4.8	33.9	0.88	5.6	33.7	2.09	4.7	33.3	3.50	5.2	33.4	7.49
76	5.0	33.5	4.8	33.9	0.88	5.5	33.6	2.08	4.8	33.1	3.60	5.5	33.0	8.06
80	4.9	33.1	4.7	33.7	0.88	5.4	33.6	2.02	4.7	33.2	3.51	5.9	33.1	8.74
84	4.7	33.4	4.5	32.8	0.84	4.9	33.0	1.85	4.9	33.0	3.67	4.9	32.5	7.43
88	4.6*	33.8	4.5	33.9	0.83	4.9	33.3	1.84	4.6	33.5	3.53	5.3	33.4	7.81
92	4.8	33.8	5.5	33.3	1.02	5.4	33.1	2.08	5.1	33.0	3.85	5.0	32.7	7.40
96	4.6	32.9	4.8	33.4	0.89	5.3	32.6	2.03	4.4	32.5	3.31	5.1	32.8	7.67
100	4.6*	33.1	4.4	33.5	0.83	4.9	33.0	1.88	4.6	32.8	3.49	5.4	33.0	8.15
104	4.9	33.5	5.6	33.5	1.07	5.1	32.8	1.96	4.8	32.7	3.61	5.6	32.4	8.59
Mean for weeks														
4-104	5.0	31.3	5.1	31.2	1.04	5.4	31.1	2.20	5.0	30.8	4.11	5.5	30.8	8.93

[a] Week indicates the last week of a 4-week interval of daily water consumption, measured weekly by cage.
[b] Dose is expressed as the mean value measured in mg/kg body weight/day.
* In the 0.0 mM acrylamide column "*" indicates a significant trend ($p < 0.05$).

TABLE 28
Water and Acrylamide Consumption by Female Mice in the 2-Year Drinking Water Study of Acrylamide

Week[a]	0 mM		0.0875 mM			0.175 mM			0.35 mM			0.70 mM		
	Water (g/day)	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose[b]	Water (g/day)	Body Weight (g)	Dose	Water (g/day)	Body Weight (g)	Dose	Water (g/day)	Body Weight (g)	Dose
4	4.7	17.2	4.6	17.7	1.72	4.5	17.3	3.40	4.4	17.1	6.80	4.6	16.8	14.26
8	4.6	18.6	4.6	18.9	1.54	4.1	18.7	2.78	4.3	18.5	6.04	4.3	18.7	11.98
12	4.2	20.5	4.4	20.5	1.37	4.2	20.4	2.65	4.3	20.3	5.38	4.2	20.1	10.77
16	4.3	21.3	4.6	21.8	1.34	4.2	21.5	2.50	4.1	21.1	4.88	4.3	21.4	10.07
20	4.1	22.4	4.4	22.8	1.22	4.2	22.6	2.33	4.1	22.0	4.73	4.6	22.1	10.47
24	4.4	22.8	4.5	23.1	1.21	4.2	22.8	2.30	4.2	22.9	4.60	4.3	22.7	9.49
28	4.5	23.4	4.4	24.0	1.14	4.2	23.7	2.21	4.2	23.1	4.58	4.4	23.5	9.32
32	4.3	24.1	4.5	24.2	1.15	4.4	24.1	2.27	4.4	23.5	4.67	4.4	23.9	9.18
36	4.3	24.1	4.7	24.9	1.19	4.4	24.4	2.24	4.3	23.8	4.47	4.3	24.3	8.77
40	4.5	24.6	4.2	24.8	1.06	4.3	24.9	2.19	4.2	24.5	4.32	4.2	24.7	8.43
44	4.2	25.5	4.3	25.8	1.04	4.2	25.7	2.06	4.2	25.1	4.15	4.1	25.6	8.21
48	4.0	25.6	4.0	25.6	0.97	4.2	25.7	2.02	4.1	25.1	4.05	4.3	25.5	8.61
52	4.2	25.6	4.0	25.8	0.97	4.1	25.7	2.01	4.0	25.2	3.96	4.2	25.3	8.22
56	4.1	26.6	4.3	26.6	1.01	4.1	25.8	1.95	3.9	25.6	3.80	4.1	25.6	7.95
60	4.4	26.1	4.1	26.7	0.96	4.5	26.2	2.17	4.2	25.9	4.14	4.4	26.0	8.48
64	4.2	26.9	4.3	27.3	0.98	4.4	27.2	2.06	4.2	26.9	3.94	4.5	26.8	8.51
68	4.4	27.6	4.2	27.5	0.95	4.5	27.4	2.09	4.1	27.2	3.78	4.5	27.5	8.25
72	4.2	27.9	4.1	28.1	0.92	4.3	27.6	1.97	4.2	27.6	3.86	4.4	28.3	7.86
76	4.3*	28.0	4.4	27.9	0.97	4.6	27.8	2.05	4.8	27.8	4.33	5.3*	28.1	9.38
80	4.2*	27.9	4.4	28.5	0.96	4.7	28.2	2.10	5.0	28.4	4.51	5.1*	28.2	9.13
84	4.5*	27.9	4.2	28.5	0.91	4.9	28.0	2.14	5.2	28.4	4.63	5.2	28.4	9.01
88	4.5*	28.7	4.4	29.3	0.95	4.7	28.7	2.06	5.3	28.8	4.81	5.5	29.1	9.38
92	4.7*	28.7	4.9	29.9	1.06	5.0	29.3	2.14	5.9	28.9	5.13	7.7*	28.9	13.63
96	4.6*	28.4	4.4	29.9	0.94	4.7	28.6	2.05	5.8	28.8	5.13	7.0*	28.7	12.36
100	4.4*	28.8	4.6	29.9	1.00	4.4	28.9	1.96	5.0	28.8	4.62	7.5*	28.6	14.03
104	4.7*	29.3	5.1	30.6	1.08	5.0	29.2	2.18	6.1	29.7	5.67	9.0*	28.9	13.62
Mean for weeks														
4-104	4.4	25.3	4.4	25.8	1.10	4.4	25.4	2.23	4.6	25.2	4.65	5.0	25.3	9.96

[a] Week indicates the last week of a 4-week interval of daily water consumption, measured weekly by cage.
[b] Dose is expressed as the mean value measured in mg/kg body weight/day.
* In the 0.0 mM acrylamide column "*" indicates a significant trend ($p < 0.05$); in the treatment column "*" indicates a significant ($p < 0.05$) pairwise comparison of the dose group to the 0.0 mM acrylamide group as determined by Dunnett's test.

Neoplastic Findings

The administration of acrylamide in the drinking water to B6C3F1 mice resulted in harderian gland neoplasms in both sexes of mice. In male mice, there was a dose-related increase in harderian gland adenoma, with the incidence being significant at all doses of acrylamide (Tables 29 and C2). Harderian gland adenocarcinoma was also observed in one male mouse administered 0.35 mM acrylamide and one male mouse administered 0.70 mM acrylamide. In female mice, harderian gland adenoma showed a dose-related increasing trend, with the incidence being significant at all doses of acrylamide (Tables 30 and D2). Morphologically, the cells in the harderian gland adenomas were cuboidal to tall columnar and generally had an abundant foamy pale cytoplasm with round to ovoid nuclei. Several patterns were noted, including papillary, cystic, and cystic papillary. Carcinomas were usually larger and associated with facial swelling or exophthalmos. Malignant tumors were highly cellular with marked pleomorphism. Mitotic figures were more evident but not abundant.

Dose-related increases in lung alveolar/bronchiolar adenoma occurred in both sexes of B6C3F1 mice, with the incidence being significant at 0.175 and 0.70 mM acrylamide in male mice (Tables 29 and C2) and at 0.35 and 0.70 mM acrylamide in female mice (Tables 30 and D2). Low incidences of alveolar/bronchiolar carcinoma (0% to 8%) were also observed in both male and female mice, but these were not considered to be related to treatment. Histomorphologically, most of the alveolar/bronchiolar adenomas were of the papillary type with tumor cells supported by a fine fibrovascular stroma that formed short projections that extended into the alveolar sacs. Tumor margins were well demarcated and compression of the surrounding tissue was distinct. Other types of growth patterns, such as solid or mixed, were present, but with a lower incidence. Carcinomas were irregular growths displaying a pleomorphic histologic pattern, with some being highly infiltrative and poorly demarcated.

Forestomach neoplasms occurred in both sexes of B6C3F1 mice administered acrylamide in the drinking water. In male mice, there were dose-related increasing trends of forestomach squamous cell papilloma, forestomach squamous cell carcinoma, and forestomach squamous cell papilloma or carcinoma (combined), with the incidence of papilloma and papilloma and carcinoma (combined) being significant in the 0.35 and 0.70 mM groups (Tables 29 and C2). Female mice demonstrated an increasing dose-related trend in forestomach squamous cell papilloma (Tables 30 and D2). Microscopically, papillomas were characterized by a solitary stalk of lamina propria protruding into the forestomach lumen, with multiple finger-like projections arising from the stalk. The epithelium covering the projections usually displayed marked hyperplasia. Squamous cell carcinomas showed proliferation into the submusosa and in some cases had features of squamous cell differentiation, such as keratin production, while others were composed of large flattened cells typical of squamous morphology.

Female B6C3F1 mice administered acrylamide in the drinking water had dose-related increasing trends in mammary gland adenoacanthoma, mammary gland adenocarcinoma, and mammary gland adenoacanthoma or adenocarcinoma (combined) (Tables 30 and D2). The incidence of mammary gland adenoacanthoma was increased significantly in the 0.70 mM group, the incidence of mammary gland adenocarcinoma was increased significantly in the 0.175 and 0.70 mM groups, and the incidence of mammary gland adenoacanthoma or adenocarcinoma (combined) was increased significantly in the 0.175, 0.35, and 0.70 mM groups (Tables 30 and D2). Mammary gland adenocarcinomas contained variably sized cystic structures lined by a pleomorphic to anaplastic cuboidal epithelium with frequent mitoses. Multiple growth patterns, such as acinar, tubular, solid, and papillary, were recognized. Adenoacanthoma showed similar features as carcinomas except that at least 25% of the tumor consisted of squamous metaplasia.

Acrylamide dosing resulted in an increasing dose-related trend in benign granulosa cell tumors of the ovary in female B6C3F1 mice, with the increase being significant at 0.70 mM acrylamide. These tumors were characterized by varying-sized follicles that compressed adjacent tissue or effaced the ovary. Follicles were composed of round to cuboidal cells arranged on a delicate basement membrane with discrete cell borders. Cell nuclei were centrally located with coarsely stippled chromatin. The cells resembled granulosa cells of normal follicles. Female mice also had dose-related increases in malignant mescenchymal skin tumors (fibrosarcoma, hemangiosarcoma, liposarcoma, myxosarcoma, neurofibrosarcoma, or sarcoma), with the incidence being significant at 0.35 and 0.70 mM acrylamide. These various mesenchymal tumors looked histologically similar to frequently noted subcutaneous neoplasms seen in B6C3F1 mice.

TABLE 29
Statistical Analysis of Selected Neoplasms in Male Mice in the 2-Year Drinking Water Study of Acrylamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Harderian Gland: Adenoma[f]					
Overall rate[a]	2/46 (4%)	13/46 (28%)	27/47 (57%)	36/47 (77%)	39/47 (83%)
Adjusted rate[b]	4.8%	30.1%	60.1%	79.9%	87.7%
Terminal rate[c]	2/39 (5%)	11/39 (28%)	22/37 (60%)	30/38 (79%)	25/28 (89%)
First incidence (days)[d]	732 (T)	610	422	551	456
Poly-3 test[e]	P<0.001	P=0.002	P<0.001	P<0.001	P<0.001
Harderian Gland: Adenocarcinoma[g]					
Overall rate	0/46 (0%)	0/46 (0%)	0/47 (0%)	1/47 (2%)	1/47 (2%)
Harderian Gland: Adenoma or Adenocarcinoma[h]					
Overall rate	2/46 (4%)	13/46 (28%)	27/47 (57%)	37/47 (79%)	39/47 (83%)
Adjusted rate	4.8%	30.1%	60.1%	82.1%	87.7%
Terminal rate	2/39 (5%)	11/39 (28%)	22/37 (60%)	31/38 (82%)	25/28 (89%)
First incidence (days)	732 (T)	610	422	551	456
Poly-3 test	P<0.001	P=0.002	P<0.001	P<0.001	P<0.001
Lung: Alveolar/Bronchiolar Adenoma[i]					
Overall rate	5/47 (11%)	6/46 (13%)	13/47 (28%)	10/45 (22%)	19/48 (40%)
Adjusted rate	11.9%	13.8%	29.9%	23.6%	47.3%
Terminal rate	5/39 (13%)	6/39 (15%)	12/37 (32%)	9/38 (24%)	14/28 (50%)
First incidence (days)	732 (T)	732 (T)	636	674	512
Poly-3 test	P<0.001	P=0.526	P=0.036	P=0.133	P<0.001
Lung: Alveolar/Bronchiolar Carcinoma[j]					
Overall rate	2/47 (4%)	0/46 (0%)	1/47 (2%)	1/45 (2%)	4/48 (8%)
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[k]					
Overall rate	6/47 (13%)	6/46 (13%)	14/47 (30%)	10/45 (22%)	20/48 (42%)
Adjusted rate	14.3%	13.8%	32.2%	23.6%	49.8%
Terminal rate	6/39 (15%)	6/39 (15%)	13/37 (35%)	9/38 (24%)	15/28 (54%)
First incidence (days)	732 (T)	732 (T)	636	674	512
Poly-3 test	P<0.001	P=0.595N	P=0.043	P=0.211	P<0.001
Stomach (Forestomach): Squamous Cell Papilloma					
Overall rate	0/46 (0%)	2/45 (4%)	2/46 (4%)	6/47 (13%)	6/44 (14%)
Adjusted rate	0%	4.7%	4.7%	13.7%	16.5%
Terminal rate	0/39 (0%)	2/39 (5%)	2/37 (5%)	5/38 (13%)	5/28 (18%)
First incidence (days)	-	732 (T)	732 (T)	502	729
Poly-3 test	P=0.002	P=0.243	P=0.242	P=0.018	P=0.009
Stomach (Forestomach): Squamous Cell Carcinoma					
Overall rate	0/46 (0%)	0/45 (0%)	0/46 (0%)	1/47 (2%)	2/44 (5%)
Adjusted rate	0%	0%	0%	2.3%	5.5%
Terminal rate	0/39 (0%)	0/39 (0%)	0/37 (0%)	1/38 (3%)	2/28 (7%)
First incidence (days)	-	-	-	732 (T)	732 (T)
Poly-3 test	P=0.024	-	-	P=0.508	P=0.209
Stomach (Forestomach): Squamous Cell Papilloma or Carcinoma[l]					
Overall rate	0/46 (0%)	2/45 (4%)	2/46 (4%)	7/47 (15%)	8/44 (18%)
Adjusted rate	0%	4.7%	4.7%	16.0%	21.9%
Terminal rate	0/39 (0%)	2/39 (5%)	2/37 (5%)	6/38 (16%)	7/28 (25%)
First incidence (days)	-	732 (T)	732 (T)	502	729
Poly-3 test	P<0.001	P=0.243	P=0.242	P=0.009	P=0.002

TABLE 29
Statistical Analysis of Selected Neoplasms in Male Mice
in the 2-Year Drinking Water Study of Acrylamide (continued)

[a] Number of animals with neoplasm per number of animals examined microscopically.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[c] Observed incidence at the terminal sacrifice.
[d] T indicates terminal sacrifice.
[e] Beneath the 0 mM acrylamide are the p values associated with the trend test. Beneath the treated group incidences are the p values corresponding to pairwise comparisons between the 0 mM acrylamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice. An N indicates a negative trend or decreased tumor incidence.
[f] The historical incidence of harderian gland adenoma in NCTR control male B6C3F1 mice is 6.0% (range 0.0% to 10.6%; Table C3a).
[g] The historical incidence of harderian gland adenocarcinoma in NCTR control male B6C3F1 mice is 0.0% (Table C3a).
[h] The historical incidence of harderian gland adenoma or adenocarcinoma in NCTR control male B6C3F1 mice is 6.0% (range 0.0% to 10.6%; Table C3a).
[i] The historical incidence of alveolar/bronchiolar adenoma of the lung in NCTR control male B6C3F1 mice is 14.4% (range 8.3% to 18.8%; Table C3b).
[j] The historical incidence of alveolar/bronchiolar carcinoma of the lung in NCTR control male B6C3F1 mice is 2.3% (range 0.0% to 8.3%; Table C3b).
[k] The historical incidence of alveolar/bronchiolar adenoma or carcinoma (combined) of the lung in NCTR control male B6C3F1 mice is 16.7% (range 10.4% to 31.3%; Table C3b).
[l] The historical incidence of squamous cell papilloma or carcinoma (combined) of the forestomach in NCTR control male B6C3F1 mice is 0.4% (range 0.0% to 2.1%; Table C3c).

TABLE 30
Statistical Analysis of Selected Neoplasms in Female Mice in the 2-Year Drinking Water Study of Acrylamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Harderian Gland: Adenoma[f]					
Overall rate[a]	0/45 (0%)	8/44 (18%)	20/48 (42%)	32/47 (68%)	31/43 (72%)
Adjusted rate[b]	0%	19.0%	44.8%	73.8%	78.8%
Terminal rate[c]	0/39 (0%)	6/35 (17%)	16/36 (44%)	18/25 (72%)	10/15 (67%)
First incidence (days)[d]	-	595	532	474	535
Poly-3 test[e]	P<0.001	P=0.003	P<0.001	P<0.001	P<0.001
Lung: Alveolar/Bronchiolar Adenoma[g]					
Overall rate	1/47 (2%)	4/47 (9%)	6/48 (13%)	11/45 (24%)	19/45 (42%)
Adjusted rate	2.2%	9.0%	13.8%	29.5%	52.7%
Terminal rate	1/39 (3%)	1/36 (3%)	4/36 (11%)	10/25 (40%)	11/15 (73%)
First incidence (days)	732 (T)	595	645	483	537
Poly-3 test	P<0.001	P=0.177	P=0.051	P<0.001	P<0.001
Lung: Alveolar/Bronchiolar Carcinoma					
Overall rate	1/47 (2%)	0/47 (0%)	0/48 (0%)	0/45 (0%)	1/45 (2%)
Mammary Gland: Adenoacanthoma[h]					
Overall rate	0/47 (0%)	1/46 (2%)	1/48 (2%)	2/45 (4%)	4/42 (10%)
Adjusted rate	0%	2.3%	2.3%	5.4%	11.6%
Terminal rate	0/39 (0%)	0/36 (0%)	0/36 (0%)	0/25 (0%)	1/15 (7%)
First incidence (days)	-	632	671	649	542
Poly-3 test	P=0.006	P=0.495	P=0.495	P=0.199	P=0.003
Mammary Gland: Adenocarcinoma[i]					
Overall rate	0/47 (0%)	4/46 (9%)	6/48 (13%)	2/45 (4%)	13/42 (31%)
Adjusted rate	0%	9.1%	13.8%	5.3%	35.6%
Terminal rate	0/39 (0%)	1/36 (3%)	4/36 (11%)	0/25 (0%)	4/15 (27%)
First incidence (days)	-	632	652	603	546
Poly-3 test	P<0.001	P=0.059	P=0.014	P=0.201	P<0.001
Mammary Gland: Adenoacanthoma or Adenocarcinoma					
Overall rate	0/47 (0%)	4/46 (9%)	7/48 (15%)	4/45 (9%)	17/42 (41%)
Adjusted rate	0%	9.1%	16.0%	10.5%	45.4%
Terminal rate	0/39 (0%)	1/36 (3%)	4/36 (11%)	0/25 (0%)	5/15 (33%)
First incidence (days)	-	625	645	596	535
Poly-3 test	P<0.001	P=0.059	P=0.007	P=0.042	P<0.001
Ovary: Benign Granulosa Cell Tumor[j]					
Overall rate	0/46 (0%)	1/45 (2%)	0/48 (0%)	1/45 (2%)	5/42 (12%)
Adjusted rate	0%	2.4%	0%	2.7%	15.4%
Terminal rate	0/39 (0%)	1/36 (3%)	0/36 (0%)	0/25 (0%)	3/15 (20%)
First incidence (days)	-	732 (T)	-	642	673
Poly-3 test	P<0.001	P=0.491	-	P=0.464	P=0.012
Skin: Fibrosarcoma, Hemangiosarcoma, Liposarcoma, Myxosarcoma, Neurofibrosarcoma, or Sarcoma[k]					
Overall rate	0/48 (0%)	0/46 (0%)	3/48 (6%)	10/45 (22%)	6/43 (14%)
Adjusted rate	0%	0%	6.9%	26.2%	17.2%
Terminal rate	0/39 (0%)	0/36 (0%)	1/36 (3%)	5/25 (20%)	0/15 (0%)
First incidence (days)	-	-	708	534	614
Poly-3 test	P<0.001	-	P=0.110	P<0.001	P=0.005

TABLE 30
Statistical Analysis of Selected Neoplasms in Female Mice in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Stomach (Forestomach): Squamous Cell Papilloma[l]					
Overall rate	4/46 (9%)	0/46 (0%)	2/48 (4%)	5/45 (11%)	8/42 (19%)
Adjusted rate	9.1%	0%	4.6%	13.3%	24.0%
Terminal rate	4/39 (10%)	0/36 (0%)	1/36 (3%)	3/25 (12%)	4/15 (27%)
First incidence (days)	732 (T)	-	692	483	583
Poly-3 test	P=0.001	P=0.063N	P=0.344N	P=0.402	P=0.070

[a] Number of animals with neoplasm per number of animals examined microscopically.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[c] Observed incidence at the terminal sacrifice.
[d] T indicates terminal sacrifice.
[e] Beneath the 0 mM acrylamide are the p values associated with the trend test. Beneath the treated group incidences are the p values corresponding to pairwise comparisons between the 0 mM acrylamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice. An N indicates a negative trend or decreased tumor incidence.
[f] The historical incidence of harderian gland adenoma in NCTR control female B6C3F1 mice is 5.3% (range 2.1% to 8.7%; Table D3c).
[g] The historical incidence of alveolar/bronchiolar adenoma of the lung in NCTR control female B6C3F1 mice is 4.7% (range 2.1% to 8.3%; Table D3a).
[h] The historical incidence of adenoacanthoma of the mammary gland in NCTR control female B6C3F1 mice is 0.4% (range 0.0% to 4.3%; Table D3b).
[i] The historical incidence of adenocarcinoma of the mammary gland in NCTR control female B6C3F1 mice is 2.9% (range 0.0% to 8.5%; Table D3b).
[j] The historical incidence of benign granulosa cell tumors of the ovaries in NCTR control female B6C3F1 mice is 0.2% (range 0.0% to 0.7%; Table D3e).
[k] The historical incidence of malignant mescenchymal skin tumors in NCTR control female B6C3F1 mice is 0.6% (range 0.0% to 8.3%) for fibrosarcoma, hemangiosarcoma, myxosarcoma, or sarcoma and 0.0% for neurofibrosarcoma (Table D3g).
[l] The historical incidence of squamous cell papilloma or carcinoma (combined) of the forestomach in NCTR control female B6C3F1 mice is 1.2% (range 0.0% to 4.3%; Table D3f).

Nonneoplastic Findings

The drinking water administration of acrylamide to B6C3F1 mice resulted in cataracts of the eyes of both sexes of mice. In male mice, the incidence of cataracts was increased in the 0.70 mM acrylamide dose group (Table 31), while in female mice the incidence was increased in both the 0.35 and 0.70 mM acrylamide dose groups (Table 32). Histopathologically, the cataracts displayed an irregular swelling of fiber cells with a granular vacuolated cytoplasm. Early mineralization was also noted along with disorganization of the lens epithelium.

Acrylamide administration resulted in a dose-related increasing trend in forestomach epithelium hyperplasia in both sexes of mice, with the incidence being significant in the 0.70 mM groups (Tables 31 and 32). Focal squamous cell hyperplasia of the forestomach was evident as having multiple finger-like projections each with its own lamina propria and with excessive keratin on the surface. Focal rather than diffuse hyperplasia was the predominant pattern.

Both sexes of B6C3F1 mice had increasing dose-related trends in hematopoietic cell proliferation of the spleen, with the incidence being significant at 0.70 mM acrylamide in male mice and 0.35 and 0.70 mM acrylamide in female mice (Tables 31 and 32). Hematopoietic cell proliferation was characterized in most animals by an increase in myeloid precursors although erythroid hyperplasia was noted occasionally.

Other nonneoplastic lesions in male B6C3F1 mice included preputial gland inflammation, which was significantly elevated at 0.35 and 0.70 mM acrylamide, and lung alveolar epithelium hyperplasia, which was significantly increased at 0.70 mM acrylamide. Alveolar epithelial hyperplasia was typically focal and did not compress the surrounding parenchyma. The cells lined a thickened alveolar septal wall, with the hyperplastic alveolar cells usually being cubordal in shape. Additional nonneoplastic lesions in female B6C3F1 mice included ovarian cysts, which were increased at 0.0875, 0.35, and 0.70 mM acrylamide.

TABLE 31
Statistical Analysis of Selected Nonneoplastic Lesions in Male Mice in the 2-Year Drinking Water Study of Acrylamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Eye					
Cataract					
Number examined microscopically	44	44	45	44	41
Overall rate[a]	3/44 (7%)	6/44 (14%)	5/45 (11%)	6/44 (14%)	9/41 (22%)
Adjusted rate[b]	7.3%	14.4%	11.7%	14.5%	25.5%
Terminal rate[c]	2/38 (5%)	6/39 (15%)	3/37 (8%)	5/37 (14%)	9/28 (32%)
First incidence (days)[d]	366	732 (T)	586	678	732 (T)
Poly-3 test[e]	P=0.023	P=0.247	P=0.378	P=0.243	P=0.029
Average severity[f]	1.3	1.3	2.0	1.3	1.3
Lung					
Alveolar Epithelium Hyperplasia					
Number examined microscopically	47	46	47	45	48
Overall rate	0/47 (0%)	0/46 (0%)	3/47 (6%)	4/45 (9%)	9/48 (19%)
Adjusted rate	0%	0%	6.9%	9.5%	22.6%
Terminal rate	0/39 (0%)	0/39 (0%)	2/37 (5%)	4/38 (11%)	7/28 (25%)
First incidence (days)	-	-	715	732 (T)	513
Poly-3 test	P<0.001	-	P=0.124	P=0.061	P=0.001
Average severity	-	-	1.7	1.8	2.1
Preputial Gland					
Inflammation					
Number examined microscopically	44	46	47	47	46
Overall rate	3/44 (7%)	6/46 (13%)	3/47 (6%)	14/47 (30%)	15/46 (33%)
Adjusted rate	7.6%	13.9%	6.8%	31.8%	38.6%
Terminal rate	3/37 (8%)	5/39 (13%)	2/37 (5%)	11/38 (29%)	10/28 (36%)
First incidence (days)	732 (T)	621	219	621	611
Poly-3 test	P<0.001	P=0.286	P=0.611N	P=0.005	P<0.001
Average severity	1.7	2.0	2.3	2.4	2.1
Spleen					
Hematopoietic Cell Proliferation					
Number examined microscopically	45	47	46	47	45
Overall rate	5/45 (11%)	6/47 (13%)	9/46 (20%)	6/47 (13%)	14/45 (31%)
Adjusted rate	12.1%	13.3%	20.0%	13.4%	34.1%
Terminal rate	5/39 (13%)	3/39 (8%)	3/37 (8%)	2/38 (5%)	6/28 (21%)
First incidence (days)	732 (T)	450	422	502	60
Poly-3 test	P=0.006	P=0.562	P=0.240	P=0.555	P=0.015
Average severity	3.0	3.0	3.3	3.3	3.3
Stomach					
Forestomach Epithelium Hyperplasia					
Number examined microscopically	46	45	46	47	44
Overall rate	0/46 (0%)	1/45 (2%)	3/46 (7%)	3/47 (6%)	8/44 (18%)
Adjusted rate	0%	2.3%	7.1%	6.9%	21.2%
Terminal rate	0/39 (0%)	1/39 (3%)	3/37 (8%)	3/38 (8%)	5/28 (18%)
First incidence (days)	-	732 (T)	732 (T)	732 (T)	512
Poly-3 test	P<0.001	P=0.506	P=0.122	P=0.126	P=0.002
Average severity	-	2.0	2.7	2.3	2.3

[a] Number of animals with lesion per number of animals examined microscopically.
[b] Poly-3 estimated lesion incidence after adjustment for intercurrent mortality.
[c] Observed incidence at the terminal sacrifice.
[d] T indicates terminal sacrifice.
[e] Beneath the 0 mM acrylamide are the p values associated with the trend test. Beneath the treated group incidences are the p values corresponding to pairwise comparisons between the 0 mM acrylamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice. An N indicates a negative trend or decreased incidence.
[f] Severity was graded as 1, minimal; 2, mild; 3, moderate; and 4, marked.

TABLE 32
Statistical Analysis of Selected Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Acrylamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Eye					
Cataract					
Number examined microscopically	45	44	47	45	38
Overall rate[a]	3/45 (7%)	2/44 (5%)	7/47 (15%)	11/45 (24%)	13/38 (34%)
Adjusted rate[b]	6.9%	4.8%	16.0%	29.8%	42.3%
Terminal rate[c]	2/39 (5%)	1/36 (3%)	6/36 (17%)	8/25 (32%)	8/15 (53%)
First incidence (days)[d]	569	595	532	708	638
Poly-3 test[e]	P<0.001	P=0.521N	P=0.155	P=0.006	P<0.001
Average severity[f]	1.3	1.5	1.9	2.2	2.4
Ovary					
Cyst					
Number examined microscopically	46	45	48	45	42
Overall rate	8/46 (17%)	18/45 (40%)	12/48 (25%)	20/45 (44%)	18/42 (43%)
Adjusted rate	18.1%	41.8%	27.8%	51.0%	52.8%
Terminal rate	8/39 (21%)	16/36 (44%)	11/36 (31%)	15/25 (60%)	9/15 (60%)
First incidence (days)	725 (T)	595	708	483	621
Poly-3 test	P=0.001	P=0.012	P=0.205	P<0.001	P<0.001
Average severity	2.5	2.5	2.9	3.2	2.9
Spleen					
Hematopoietic Cell Proliferation					
Number examined microscopically	46	46	48	45	44
Overall rate	5/46 (11%)	10/46 (22%)	6/48 (13%)	14/45 (31%)	18/44 (41%)
Adjusted rate	11.3%	22.2%	13.7%	35.1%	47.8%
Terminal rate	4/39 (10%)	5/36 (14%)	2/36 (6%)	4/25 (16%)	2/15 (13%)
First incidence (days)	554	595	639	483	535
Poly-3 test	P<0.001	P=0.136	P=0.492	P=0.008	P<0.001
Average severity	2.8	3.5	2.8	3.4	3.3
Stomach					
Forestomach Epithelium Hyperplasia					
Number examined microscopically	46	46	48	45	42
Overall rate	5/46 (11%)	9/46 (20%)	4/48 (8%)	4/45 (9%)	11/42 (26%)
Adjusted rate	11.4%	20.7%	9.0%	10.9%	31.5%
Terminal rate	5/39 (13%)	7/36 (19%)	2/36 (6%)	4/25 (16%)	3/15 (20%)
First incidence (days)	732 (T)	628	532	732 (T)	539
Poly-3 test	P=0.026	P=0.185	P=0.494N	P=0.612N	P=0.025
Average severity	2.6	2.2	2.3	2.3	2.3

[a] Number of animals with lesion per number of animals examined microscopically.
[b] Poly-3 estimated lesion incidence after adjustment for intercurrent mortality.
[c] Observed incidence at the terminal sacrifice.
[d] T indicates terminal sacrifice.
[e] Beneath the 0 mM acrylamide are the p values associated with the trend test. Beneath the treated group incidences are the p values corresponding to pairwise comparisons between the 0 mM acrylamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice. An N indicates a negative trend or decreased incidence.
[f] Severity was graded as 1, minimal; 2, mild; 3, moderate; and 4, marked.

DISCUSSION AND CONCLUSIONS

In this study, male and female F344/N rats and B6C3F1 mice were exposed to acrylamide in the drinking water for 2 years. In both sexes of F344/N rats, there were significant increases in the incidences of thyroid gland neoplasms. Male F344/N rats also had significant dose-related increases in testicular, heart, and pancreatic neoplasms, while female F344/N rats demonstrated significant increases in clitoral gland, mammary gland, oral cavity, and skin neoplasms. In both sexes of B6C3F1 mice, there were significant dose-related increases in neoplasms of the harderian gland, lung, and forestomach. Female B6C3F1 mice also had significant dose-related increases in mammary gland, ovary, and skin neoplasms.

Acrylamide is metabolized to glycidamide (Calleman *et al*., 1990), which reacts with DNA to give to a number of DNA adducts, primarily N7-GA-Gua and N3-GA-Ade (Figure 3; Segerbäck *et al*., 1995; Solomon, 1999; Gamboa da Costa *et al*., 2003). The oxidation of acrylamide to glycidamide is catalyzed by cytochrome P450 2E1 (Sumner *et al*., 1999; Ghanayem *et al*., 2005a), and mice devoid of cytochrome P450 2E1 and administered acrylamide have much reduced levels of male germ cell mutagenicity (Ghanayem *et al*., 2005b), micronuclei (Ghanayem *et al*., 2005c), and glycidamide DNA adducts (Ghanayem *et al*., 2005a) compared to their wild-type counterparts. In addition, adult Big Blue mice and rats and neonatal B6C3F1/*Tk*$^{+/-}$ mice treated with acrylamide have increases in mutant frequencies, comparable to those induced by glycidamide (Manjanatha *et al*., 2006; Guo *et al*., 2009; Mei *et al*., 2010; Von Tungeln *et* al., 2009; Wang *et al*., 2010), and the pattern of mutations induced by acrylamide in mice is consistent with the types of mutations and DNA adducts arising from glycidamide (Manjanatha *et al*., 2006). These data indicate that acrylamide is genotoxic through conversion to glycidamide. As discussed below, the results of the current bioassays lend support to the concept that acrylamide is carcinogenic through a similar pathway.

The strongest response for neoplasm induction in F344/N rats was in the mammary gland of females, where acrylamide induced a significant increase in fibroadenoma (Table 17). Mammary gland fibro-adenoma has been detected in previous bioassays of acrylamide conducted in female F344 rats (Johnson *et al*., 1986; Friedman *et al*., 1995). In the Johnson *et al*. (1986) study, a statistically significant increase in the incidence of mammary gland fibroma or fibroadenoma occurred at 2 mg acrylamide per kg body weight per day, whereas in the Friedman *et al*. (1995) study, a significant increase in mammary gland fibro-adenoma was observed at 1 and 3 mg acrylamide per kg body weight per day (Table 33). By comparison, in the current study, a statistically significant increase in mammary gland fibroadenoma occurred at 0.175 mM acrylamide (Table 33), which was equivalent to a dose of 0.88 mg acrylamide per kg body weight per day. Furthermore, the incidence of fibroadenoma in all acrylamide dose groups, with the exception of the 0.0875 mM group, exceeded the historical range observed in control female F344/N rats in previous experiments at the NCTR (27.1% to 42.6%; Table B3c).

Although there has been controversy regarding the mechanism of induction of mammary tumors in F344/N rats administered acrylamide (Park *et al*., 2002; Klaunig and Kamendulis, 2005), female F344/N rats treated with a single intraperitoneal dose of acrylamide have substantial levels of N7-GA-Gua in their mammary gland DNA (Doerge *et al*., 2005c). Even higher levels of N7-GA-Gua were obtained upon dosing with an equimolar amount of glycidamide, which is probably a reflection of less efficient formation of glycidamide in rats (as compared to mice at high doses of acrylamide). These data indicate that the conversion of acrylamide to glycidamide plays an important role in the induction of mammary gland tumors in female F344/N rats.

Acrylamide induced thyroid gland follicular cell adenoma or carcinoma in both male and female F344/N rats. In both sexes, the incidence of thyroid gland follicular cell adenoma or carcinoma was significantly increased at 0.70 mM acrylamide (2.7 and 4.0 mg acrylamide per kg body per day for males and females, respectively; Tables 16 and 17). In male F344/N rats, the incidence in all dosed groups exceeded the historical range observed in control male F344/N rats in previous experiments at the NCTR (0.0% to 2.1%; Table A3a), while in female F344/N rats, the incidence in all dosed groups except 0.0875 mM acrylamide exceeded the historical control range (0.0% to 2.9%; Table B3a).

TABLE 33
Comparative Tumor Incidence in Male and Female F344 Rats Treated Chronically for 2 Years with Acrylamide

Neoplasm	Sex	Acrylamide Dose	Incidence	Reference
Mammary gland adenoma, fibroadenoma, or fibroma	Female	0 mg/kg bw	10/60 (17%)	Johnson *et al*., 1986
		0.01 mg/kg bw	11/60 (18%)	
		0.1 mg/kg bw	9/60 (15%)	
		0.5 mg/kg bw	19/58 (33%)	
		2.0 mg/kg bw	23/61 (38%)[a]	
Mammary gland fibroadenoma	Female	0 mg/kg bw	5/46 (11%)	Friedman *et al*., 1995
		0 mg/kg bw	4/50 (8%)	
		1.0 mg/kg bw	20/94 (21%)[b]	
		3.0 mg/kg bw	26/95 (27%)[b]	
Mammary gland fibroadenoma	Female	0 mM (0 mg/kg bw)	16/48 (33%)	This study
		0.0875 mM (0.44 mg/kg bw)	18/48 (38%)	
		0.175 mM (0.88 mg/kg bw)	24/46 (52%)[c]	
		0.35 mM (1.84 mg/kg bw)	22/47 (47%)[c]	
		0.70 mM (4.02 mg/kg bw)	31/48 (65%)[c]	
Thyroid gland follicular cell adenoma or carcinoma	Female	0 mg/kg bw	1/58 (2%)	Johnson *et al*., 1986
		0.01 mg/kg bw	0/59 (0%)	
		0.1 mg/kg bw	1/59 (2%)	
		0.5 mg/kg bw	1/58 (2%)	
		2.0 mg/kg bw	5/60 (8%)[a]	
Thyroid gland follicular cell adenoma or carcinoma	Female	0 mg/kg bw	1/50 (2%)	Friedman *et al*., 1995
		0 mg/kg bw	1/50 (2%)	
		1.0 mg/kg bw	10/100 (10%)	
		3.0 mg/kg bw	23/100 (23%)[b]	
Thyroid gland follicular cell adenoma or carcinoma	Female	0 mM (0 mg/kg bw)	0/48 (0%)	This study
		0.0875 mM (0.44 mg/kg bw)	0/48 (0%)	
		0.175 mM (0.88 mg/kg bw)	2/48 (4%)	
		0.35 mM (1.84 mg/kg bw)	3/48 (6%)	
		0.70 mM (4.02 mg/kg bw)	4/47 (9%)[c]	
Thyroid gland follicular cell adenoma	Male	0 mg/kg bw	1/60 (2%)	Johnson *et al*., 1986
		0.01 mg/kg bw	0/58 (0%)	
		0.1 mg/kg bw	2/59 (3%)	
		0.5 mg/kg bw	1/59 (2%)	
		2.0 mg/kg bw	7/59 (12%)[a]	
Thyroid gland follicular cell adenoma	Male	0 mg/kg bw	2/100 (2%)	Friedman *et al*., 1995
		0 mg/kg bw	1/102 (1%)	
		0.1 mg/kg bw	9/203 (4%)	
		0.5 mg/kg bw	5/101 (5%)	
		2.0 mg/kg bw	12/75 (16%)[b]	

TABLE 33
Comparative Tumor Incidence in Male and Female F344 Rats Treated Chronically for 2 Years with Acrylamide (continued)

Neoplasm	Sex	Acrylamide Dose	Incidence	Reference
Thyroid gland follicular cell adenoma or carcinoma	Male	0 mM (0 mg/kg bw)	1/47 (2%)	This study
		0.0875 mM (0.33 mg/kg bw)	3/48 (6%)	
		0.175 mM (0.66 mg/kg bw)	4/47 (9%)	
		0.35 mM (1.32 mg/kg bw)	6/48 (13%)	
		0.70 mM (2.71 mg/kg bw)	9/48 (19%)*	
Mesothelioma of the testes tunica albuginea	Male	0 mg/kg bw	3/60 (5%)	Johnson *et al*., 1986
		0.01 mg/kg bw	0/60 (0%)	
		0.1 mg/kg bw	7/60 (12%)	
		0.5 mg/kg bw	11/60 (18%)[a]	
		2.0 mg/kg bw	10/60 (17%)[a]	
Mesothelioma of the testes tunica	Male	0 mg/kg bw	4/102 (4%)	Friedman *et al*., 1995
		0 mg/kg bw	4/102 (4%)	
		0.1 mg/kg bw	9/204 (4%)	
		0.5 mg/kg bw	8/102 (8%)	
		2.0 mg/kg bw	13/75 (17%)[b]	
Malignant mesothelioma of the epididymis or testes tunica vaginalis	Male	0 mM (0 mg/kg bw)	2/48 (4%)	This study
		0.0875 mM (0.33 mg/kg bw)	2/48 (4%)	
		0.175 mM (0.66 mg/kg bw)	1/48 (2%)	
		0.35 mM (1.32 mg/kg bw)	5/48 (10%)	
		0.70 mM (2.71 mg/kg bw)	8/48 (17%)[c]	

[a] A statistical difference from control group, mortality adjustment via Mantel-Haenszel procedure as described by Peto (1974), $\alpha = 0.05$.
[b] Statistically significant, $p<0.001$ using the method described by Peto *et al*. (1980).
[c] Statistically significant as assessed by continuity-corrected Poly-3 tests (Bailer and Portier, 1988), as modified by Bieler and Williams (1993).

Follicular cell carcinoma was detected in all dosed groups of F344/N rats (Tables 16 and 17), except the females administered 0.0875 mM acrylamide. Follicular cell carcinoma has not been observed in either male (Table A3a) or female (Table B3a) control groups in previous studies at the NCTR.

Acrylamide-induced thyroid gland follicular cell adenoma or carcinoma has been reported previously in F344 rats given acrylamide (Johnson *et al*., 1986; Friedman et al., 1995). In the Johnson *et al*. (1986) study, a statistically significant increase in the incidences of follicular cell adenoma or combined adenoma or carcinoma occurred in males and females, respectively, at 2 mg acrylamide per kg body weight per day (Table 33). The same was true in the Friedman *et al*. (1995) study for male rats, whereas in female F344 rats, a significant increase in follicular cell adenoma or carcinoma (combined) occurred at 3 mg acrylamide per kg body weight per day.

N7-GA-Gua has been detected in thyroid gland DNA from both sexes of F344/N rats treated with acrylamide or glycidamide (Doerge *et al*., 2005c); thus, as with mammary gland tumors, the combined data are consistent with the conversion of acrylamide to glycidamide as being an important step in the induction of thyroid follicular cell tumors in F344/N rats.

Previous chronic bioassays of acrylamide in male F344 rats have demonstrated the induction of mesotheliomas on the tunica vaginalis of the testes, with statistically significant increases being observed at 0.5 and 2.0 mg acrylamide per kg body weight per day in the Johnson *et al*. (1986) study and at 2 mg acrylamide per kg body weight per day in the Friedman *et al*. (1995) study (Table 33). In the current study, the administration of acrylamide was associated with the development of malignant mesothelioma on the membranes surrounding the epididymis and on the testicular tunica, with the neoplasm being observed more commonly in the epididymis than on the testes. The incidence of mesothelioma was significant at 0.70 mM acrylamide (2.7 mg acrylamide per kg body weight per day) (Table 16), and the incidences at 0.35 and 0.70 mM acrylamide (10% and 17%, respectively) exceeded the historical range for mesothelioma in all organs observed in control male F344/N rats in experiments conducted at the NCTR (3.3% to 6.4%; Table A3b).

As with the other tumor sites discussed previously, N7-GA-Gua has been detected in DNA from the testes of F344/N rats treated with acrylamide or glycidamide, with the levels being higher than those found in any of the other tissues (Doerge *et al*., 2005c).

In addition to the neoplasms noted above, the administration of acrylamide resulted in significant increases in heart malignant schwannoma and pancreatic islets adenoma in male F344/N rats (Table 16) and clitoral gland carcinoma, oral cavity neoplasms, and skin neoplasms in female F344/N rats (Table 17). The incidence of the malignant schwannoma was significant in the 0.70 mM group, and the lesion was detected in all groups (including the control group) of male F344/N rats (Table 16), as well as in nearly all groups (including the control group) of female F344/N rats (Table B2). Malignant heart schwannoma has not been observed previously in control male or female F344/N rats from experiments conducted at the NCTR (Tables A3c and B3f), nor was this neoplasm reported in previous bioassays of acrylamide in F344/N rats (Johnson *et al*., 1986; Friedman *et al*., 1995).

The administration of acrylamide was associated with a dose-related increase in pancreatic islet adenoma in male F344/N rats, with the incidence being significant in the 0.70 mM group (Table 16). Pancreatic islet tumors were not reported in previous bioassays of acrylamide in F344 rats (Johnson *et al*., 1986; Friedman *et al*., 1995), and the incidence observed in the 0.70 mM group (13%) is within the historical control range for control male F344/N rats in studies conducted at the NCTR (4.2% to 13.1%; Table A3d). As noted in the Introduction, occupational, but not dietary, exposure to acrylamide may be associated with pancreatic cancer in humans.

Four types of clitoral gland epithelial neoplasms (adenomas, carcinomas, squamous cell papillomas, and squamous cell carcinomas) were observed in the female F344/N rats. Of these neoplasms, acrylamide caused dose-related trends in clitoral gland squamous cell papilloma and carcinoma, with the incidence of clitoral gland carcinoma being significantly increased in the 0.0875, 0.175, and 0.70 mM groups (Table 17). The incidence of clitoral gland carcinoma in these dosed groups also exceeded that observed in control female F344/N rats from previous bioassays conducted at the NCTR (0% to 10.4%; Table B3b).

Squamous cell neoplasms of the oral cavity (oral mucosa or tongue) also occurred in female F344/N rats, with the incidence being significant in the 0.70 mM group (Table 17). Several of the neoplasms originated in the tongue, but other locations in the oral cavity, most commonly the hard palate, were affected more often. The incidence of oral cavity squamous cell papilloma or carcinoma in all acrylamide dosed groups of female F344/N rats, with the exception of the 0.175 mM group,

exceeded that observed in control female F344/N rats from previous bioassays conducted at the NCTR (0% to 2.1%; Table B3d). Oral cavity squamous cell neoplasms were also observed in male F344/N rats; however, neither the dose-related trends nor incidences were significant.

Female F344/N rats had dose-related increases in skin mesenchymal tumors, with the incidence in the 0.70 mM acrylamide group (10%) exceeding the historical range for mesenchymal tumors in control female F344/N rats in experiments conducted at the NCTR (0.0% to 2.1%; Table B3e).

Female F344/N rats administered acrylamide in the drinking water had dose-related increases in hepatocellular adenoma (Table 17). Although the incidences of hepatocellular adenoma did not reach statistical significance, that in the 0.70 mM group (6%) exceeded the historical range for hepatocellular adenoma observed in control female F344/N rats in experiments conducted at the NCTR (0.0% to 2.1%; Table B3g). As with the other tissues discussed above, N7-GA-Gua has been detected in liver DNA from F344 rats treated with acrylamide or glycidamide (Segerbäck *et al.*, 1995; Doerge *et al*., 2005b,c; Manière *et al*., 2005; Tareke *et al*., 2006).

In the Johnson *et al*. (1986) bioassay with acrylamide, tumors of glial cell origin were detected in the brain and spinal cord of male and female F344 rats, with the incidence being significant at the highest dose of 2.0 mg acrylamide per kg body weight per day in females. This observation was not repeated in a subsequent study in which an even higher dose of acrylamide (3.0 mg acrylamide per kg body weight per day) was administered (Friedman *et al*., 1995). Because of this dichotomy, special attention was given to the brain and spinal cord during the histopathological examinations (see the Materials and Methods section for a description of the multiple layers of review). A few proliferative glial cell lesions, in particular astrocytomas and gliosis, were detected; however, neither showed dose-related trends or statistically significant incidences in either male or female F344/N rats.

A nonneoplastic lesion associated with acrylamide treatment was axonal degeneration of the sciatic nerve, which occurred in a dose-related manner in both sexes of rats, with the incidence being significant at 0.70 mM acrylamide (Tables 18 and 19). Peripheral nerve degeneration was also present in both sexes of rats administered 3.52 mM acrylamide in the 3-month study (Table 9). The rats in the 3-month study also displayed hind-limb paralysis, a condition not observed in the 2-year study. Peripheral nerve degeneration was reported by Johnson *et al*. (1986) and Friedman *et al*. (1995) in their 2 year bioassays with acrylamide in F344 rats.

The strongest response for neoplasm induction in the B6C3F1 mice was the harderian gland. In male B6C3F1 mice, the incidence of harderian gland adenoma increased from 4% in the control group to 83% in mice receiving 0.70 mM acrylamide in the drinking water (Table 29) and in female B6C3F1 mice, the incidence of harderian gland adenoma increased from 0% in the control group to 72% in mice administered 0.70 mM acrylamide (Table 30). Furthermore, even at the lowest dose of acrylamide (0.0875 mM acrylamide), the incidence of neoplasms in the harderian gland [28% in male mice and 18% in female mice] exceeded the range observed in control male (0.0% to 10.6%; Table C3a) and female (2.1% to 8.7%; Table D3c) B6C3F1 mice in previous studies conducted at the NCTR.

The small size of the harderian gland precluded DNA adduct analyses in this tissue. Nonetheless, the harderian gland has been a target tissue for other low-molecular-weight carcinogens (*e.g.*, *N*-methylol-acrylamide, 1,3-butadiene, isoprene, chloroprene, and urethane) thought to be metabolized to electrophilic epoxides (Bucher *et al*., 1990; Melnick and Sills, 2001; Beland *et al*., 2005), which is consistent with the concept that acrylamide is activated through metabolism to glycidamide. Nonneoplastic lesions were also detected in the eyes of both sexes of B6C3F1 mice. These included cataracts of the cornea (Tables 31 and 32), which may be a consequence of the impairment of the harderian gland due to tumor formation.

In both sexes of B6C3F1 mice, there were significant dose-dependent increases in lung alveolar/bronchiolar adenoma (Tables 29 and 30). Nonneoplastic changes included lung alveolar epithelial hyperplasia in male mice (Table 31). In male B6C3F1 mice, the incidence of combined lung alveolar/bronchiolar adenoma or carcinoma in the 0.70 mM group (42%) exceeded the range observed in control male B6C3F1 mice in previous studies at the NCTR (10.4% to 31.3%; Table C3b); the same is true for the incidences of lung alveolar/bronchiolar adenoma in female B6C3F1 mice administered 0.175, 0.35, and 0.70 mM acrylamide (13%, 24%, and 42%, respectively) (historical control range = 2.1% to 8.3% in female B6C3F1 mice; Table D3a).

As with harderian gland neoplasms, lung alveolar/ bronchiolar adenoma and carcinoma have been observed in B6C3F1 mice treated with other low-molecular-weight carcinogens that are thought to be

metabolized to electrophilic epoxides (Bucher *et al*., 1990; Melnick and Sills, 2001; Beland *et al*., 2005), which supports the concept that acrylamide is activated through metabolism to glycidamide. Further support for this premise is afforded by DNA adduct analyses that have been conducted in B6C3F1 mice and other strains of mice (Gamboa da Costa *et al*., 2003; Doerge *et al*., 2005c; Ghanayem *et al*., 2005a; Von Tungeln *et al*., 2009). In all cases, high levels of N7-GA-Gua have been detected in lung DNA, accompanied by lower, but still substantial, levels of N3-GA-Ade. Furthermore, the levels of both DNA adducts increased with the dose of acrylamide (Gamboa da Costa *et al*., 2003; Von Tungeln *et al*., 2009) and with time upon repeated administration (Doerge *et al*., 2005c). Lung tumors have also been detected in other strains of mice administered acrylamide, including male and female A/J mice (Bull *et al*., 1984a), female Swiss-ICR mice (Bull *et al*., 1984b), and female SENCAR mice (Robinson *et al*., 1986).

Stomach (forestomach) squamous cell papilloma or carcinoma was observed in male B6C3F1 mice, with the increase being significant at the two highest doses of acrylamide (Table 29). Even at the lowest dose of 0.0875 mM acrylamide, the incidence of forestomach squamous cell papilloma or carcinoma (combined) (4%) exceeded the range observed in control male B6C3F1 mice in previous experiments at the NCTR (0.0% to 2.1%; Table C3c). A dose-related increasing trend of forestomach squamous cell papilloma also occurred in female B6C3F1 mice administered acrylamide (Table 30). Although the increase in the incidence of forestomach papilloma did not reach statistical significance, the levels detected in the 0.35 and 0.70 mM acrylamide groups (11% and 19%, respectively) exceeded the range observed in control female B6C3F1 mice for forestomach papilloma or carcinoma (combined) in previous experiments at the NCTR (0.0% to 4.3%; Table D3f). In addition to forestomach squamous cell papilloma or carcinoma, dose-related increases in forestomach epithelium hyperplasia were present in both sexes of B6C3F1 mice (Tables 31 and 32).

Squamous cell tumors of the forestomach have been observed in B6C3F1 mice administered 1,3-butadiene, isoprene, chloroprene, and urethane (Melnick and Sills, 2001; Beland *et al*., 2005). In some instances, male B6C3F1 mice have appeared to be more sensitive to the carcinogenic effects (*e.g*., urethane), while in other bioassays, female B6C3F1 mice have appeared to be more susceptible (*e.g*., 1,3-butadiene).

Other neoplasms observed in female B6C3F1 mice administered acrylamide included mammary gland adenoacanthoma/adenocarcinoma, benign ovarian granulosa cell tumors, and various types of skin sarcomas (Table 30). Similar types of mammary gland and/or ovarian tumors have been induced in female B6C3F1 mice treated with *N*-methylolacrylamide, 1,3-butadiene, chloroprene, and urethane (Bucher *et al*., 1990; Melnick and Sills, 2001; Beland *et al*., 2005).

Liver DNA from mice administered acrylamide contains N7-GA-Gua and N3-GA-Ade, at levels that are comparable to those found in other tumor target tissues (Gamboa da Costa *et al*., 2003; Doerge *et al*., 2005c; Ghanayem *et al*., 2005a; Von Tungeln *et al*., 2009), and Big Blue mice treated with acrylamide have increased hepatic mutant frequencies (Manjanatha *et al*., 2006). Chemicals similar to acrylamide, such as urethane, *N*-methylolacrylamide, 1,3-butadiene, and chloroprene, have been associated with an increased incidence of hepatocellular adenoma or carcinoma in male and/or female B6C3F1 mice (Bucher *et al*., 1990; Melnick and Sills, 2001; Beland *et al*., 2005). These results suggested that liver of both sexes of B6C3F1 mice would be a potential tumor target tissue for acrylamide. Nonetheless, acrylamide did not increase the liver neoplasm incidence in male B6C3F1 mice, and although female B6C3F1 mice administered acrylamide did demonstrate a significant dose-related increasing trend in hepatocellular adenoma (Table D2), the incidence at the highest dose of 0.70 mM acrylamide (11%) was not significant and exceeded only slightly that of the historical range observed in control female B6C3F1 mice in previous studies at the NCTR (0.0% to 10.6%; Table D3d).

CONCLUSIONS

Under the conditions of these 2-year drinking water studies, there was *clear evidence of carcinogenic activity* of acrylamide in male F344/N rats based on increased incidences of malignant mesothelioma of the epididymis and testis tunica, malignant schwannoma of the heart, and follicular cell adenoma or carcinoma of the thyroid gland. An increased incidence of pancreatic islet adenoma was also considered related to acrylamide exposure.

There was *clear evidence of carcinogenic activity* of acrylamide in female F344/N rats based on increased incidences of fibroadenoma of the mammary gland, squamous cell neoplasms (primarily papilloma) of the

oral cavity (mucosa or tongue), mesenchymal neoplasms (fibroma, fibrosarcoma, or sarcoma) of the skin, and follicular cell neoplasms (adenoma or carcinoma) of the thyroid gland. Increased incidences of hepatocellular adenoma of the liver and carcinoma of the clitoral gland were also considered to be related to acrylamide exposure. The occurrence of malignant schwannoma of the heart may have been related to acrylamide exposure.

There was *clear evidence of carcinogenic activity* of acrylamide in male B6C3F1 mice based on increased incidences of neoplasms (primarily adenoma) of the harderian gland, alveolar/bronchiolar neoplasms (primarily adenoma) of the lung and squamous cell neoplasms (primarily papilloma) of the forestomach.

There was *clear evidence of carcinogenic activity* of acrylamide in female B6C3F1 mice based on increased incidences of harderian gland adenoma, alveolar/bronchiolar adenoma of the lung, adenoacanthoma and adenocarcinoma of the mammary gland, benign granulosa cell neoplasms of the ovary, and malignant mesenchymal neoplasms of the skin. Increased incidences of squamous cell papilloma of the forestomach were also considered to be related to acrylamide exposure.

Exposure to acrylamide was associated with increased incidences of degeneration of the retina and sciatic nerve in male and female rats; preputial gland duct ectasia in male rats; adrenal cortex hypertrophy and cytoplasmic vacuolization, bone marrow hyperplasia, ovarian atrophy, and spleen hematopoietic cell proliferation in female rats; cataracts of the eye, spleen hematopoietic cell proliferation, and forestomach epithelial hyperplasia in male and female mice; preputial gland inflammation and lung epithelial hyperplasia in male mice; and ovarian cysts in female mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 13. A summary of the Peer Review Panel comments and the public discussion on this Technical Report appears on page 15.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF ACRYLAMIDE

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Drinking Water Study of Acrylamide[a]

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Disposition Summary					
Animals initially in study	48	48	48	48	48
Early deaths					
Moribund sacrifice	18	20	20	23	27
Natural deaths	6	4	2		4
Survivors					
Moribund sacrifice	5	9	7	7	7
Natural deaths	2	1		2	1
Terminal sacrifice	17	14	19	16	9
Animals examined microscopically	48	48	48	48	48
Alimentary System					
Intestine large, cecum	(45)	(48)	(47)	(48)	(47)
Leukemia mononuclear		4 (8%)	2 (4%)	2 (4%)	
Mesothelioma malignant	1 (2%)	1 (2%)			
Intestine large, colon	(45)	(47)	(47)	(48)	(48)
Leukemia mononuclear	1 (2%)			1 (2%)	
Lymphoma malignant				1 (2%)	
Mesothelioma malignant	1 (2%)				
Intestine small, duodenum	(45)	(48)	(47)	(48)	(48)
Leukemia mononuclear		1 (2%)			
Mesothelioma malignant	1 (2%)	1 (2%)			
Intestine small, ileum	(44)	(47)	(47)	(48)	(47)
Leukemia mononuclear		3 (6%)	1 (2%)		3 (6%)
Mesothelioma malignant	1 (2%)				
Intestine small, jejunum	(43)	(46)	(46)	(48)	(45)
Carcinoma					1 (2%)
Leukemia mononuclear				1 (2%)	
Mesothelioma malignant	1 (2%)				
Liver	(48)	(48)	(48)	(48)	(48)
Hepatocellular adenoma	2 (4%)		3 (6%)	2 (4%)	1 (2%)
Hepatocellular adenoma, multiple			1 (2%)		
Hepatocellular carcinoma			1 (2%)		
Histiocytic sarcoma		1 (2%)	1 (2%)	1 (2%)	
Leukemia mononuclear	23 (48%)	20 (42%)	20 (42%)	29 (60%)	26 (54%)
Mesothelioma malignant	1 (2%)				
Mesentery	(2)	(4)	(7)	(7)	(4)
Carcinoma, metastatic, intestine small, jejunum					1 (25%)
Leukemia mononuclear	1 (50%)	1 (25%)	1 (14%)	2 (29%)	1 (25%)
Mesothelioma malignant	1 (50%)	1 (25%)			
Oral mucosa	(0)	(0)	(2)	(6)	(3)
Squamous cell carcinoma			1 (50%)	1 (17%)	
Squamous cell papilloma				3 (50%)	1 (33%)
Pancreas	(46)	(48)	(48)	(48)	(48)
Histiocytic sarcoma				1 (2%)	
Leukemia mononuclear	2 (4%)	6 (13%)	3 (6%)	6 (13%)	5 (10%)
Lymphoma malignant				1 (2%)	
Mesothelioma malignant		1 (2%)			
Acinar cell, adenoma					2 (4%)
Salivary glands	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear	1 (2%)	1 (2%)	1 (2%)		
Lymphoma malignant				1 (2%)	
Stomach, forestomach	(47)	(48)	(47)	(48)	(48)
Leukemia mononuclear			2 (4%)	1 (2%)	
Lymphoma malignant				1 (2%)	
Squamous cell papilloma		1 (2%)			
Stomach, glandular	(47)	(48)	(47)	(48)	(48)
Leukemia mononuclear		1 (2%)	3 (6%)		
Lymphoma malignant				1 (2%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Alimentary System (continued)					
Tongue	(3)	(1)	(0)	(2)	(1)
Squamous cell carcinoma					1 (100%)
Squamous cell papilloma	1 (33%)	1 (100%)		1 (50%)	
Cardiovascular System					
Blood vessel	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear		2 (4%)		1 (2%)	
Heart	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear	7 (15%)	9 (19%)	9 (19%)	15 (31%)	10 (21%)
Lymphoma malignant				1 (2%)	
Schwannoma malignant	1 (2%)	2 (4%)	3 (6%)	4 (8%)	6 (13%)
Endocrine System					
Adrenal cortex	(48)	(48)	(48)	(48)	(48)
Carcinoma			1 (2%)		
Leukemia mononuclear	1 (2%)	3 (6%)	3 (6%)	2 (4%)	3 (6%)
Mesothelioma malignant	1 (2%)				
Adrenal medulla	(48)	(48)	(47)	(48)	(47)
Leukemia mononuclear	2 (4%)	6 (13%)	8 (17%)	3 (6%)	7 (15%)
Pheochromocytoma benign	6 (13%)	4 (8%)	5 (11%)	9 (19%)	
Pheochromocytoma malignant		3 (6%)	6 (13%)	2 (4%)	2 (4%)
Bilateral, pheochromocytoma benign	2 (4%)			1 (2%)	1 (2%)
Bilateral, pheochromocytoma malignant					1 (2%)
Islets, pancreatic	(46)	(48)	(48)	(48)	(48)
Adenoma	1 (2%)	2 (4%)	4 (8%)	1 (2%)	6 (13%)
Carcinoma				1 (2%)	
Leukemia mononuclear	1 (2%)		1 (2%)		
Parathyroid gland	(46)	(48)	(47)	(47)	(44)
Adenoma					1 (2%)
Pituitary gland	(48)	(48)	(47)	(48)	(47)
Leukemia mononuclear		1 (2%)	2 (4%)	3 (6%)	4 (9%)
Lymphoma malignant				1 (2%)	
Pars distalis, adenoma	21 (44%)	31 (65%)	24 (51%)	31 (65%)	28 (60%)
Thyroid gland	(47)	(48)	(47)	(48)	(48)
Lymphoma malignant				1 (2%)	
C-cell, adenoma	2 (4%)		5 (11%)	2 (4%)	3 (6%)
C-cell, carcinoma		1 (2%)			1 (2%)
Follicular cell, adenoma		1 (2%)	1 (2%)	1 (2%)	3 (6%)
Follicular cell, carcinoma	1 (2%)	2 (4%)	3 (6%)	6 (13%)	6 (13%)
General Body System					
Peritoneum	(0)	(0)	(0)	(0)	(2)
Tunica vaginalis, mesothelioma malignant					2 (100%)
Tissue NOS	(0)	(1)	(1)	(1)	(1)
Abdominal, carcinoma, metastatic, adrenal cortex			1 (100%)		
Genital System					
Epididymis	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear		2 (4%)			1 (2%)
Mesothelioma malignant	2 (4%)	2 (4%)	1 (2%)	5 (10%)	8 (17%)
Penis	(1)	(0)	(0)	(1)	(1)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Genital System (continued)					
Preputial gland	(48)	(47)	(48)	(48)	(48)
Adenoma		3 (6%)	3 (6%)	1 (2%)	4 (8%)
Adenoma, multiple			1 (2%)		
Carcinoma	7 (15%)	3 (6%)	4 (8%)	4 (8%)	2 (4%)
Leukemia mononuclear	1 (2%)	1 (2%)	1 (2%)		
Lymphoma malignant				1 (2%)	
Squamous cell carcinoma	4 (8%)	1 (2%)	1 (2%)	5 (10%)	
Squamous cell papilloma	1 (2%)		1 (2%)	2 (4%)	
Prostate	(47)	(48)	(48)	(48)	(48)
Histiocytic sarcoma		1 (2%)			
Leukemia mononuclear	2 (4%)	2 (4%)	2 (4%)	2 (4%)	1 (2%)
Lymphoma malignant				1 (2%)	
Mesothelioma malignant		1 (2%)			
Seminal vesicle	(48)	(48)	(47)	(48)	(48)
Leukemia mononuclear		2 (4%)	2 (4%)	1 (2%)	1 (2%)
Lymphoma malignant				1 (2%)	
Mesothelioma malignant	1 (2%)	1 (2%)			
Testes	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear		1 (2%)	1 (2%)		
Lymphoma malignant				1 (2%)	
Mesothelioma malignant	1 (2%)	2 (4%)	1 (2%)	1 (2%)	5 (10%)
Bilateral, interstitial cell, adenoma	23 (48%)	19 (40%)	26 (54%)	23 (48%)	24 (50%)
Interstitial cell, adenoma	13 (27%)	13 (27%)	10 (21%)	14 (29%)	9 (19%)
Hematopoietic System					
Bone marrow	(47)	(48)	(48)	(48)	(48)
Histiocytic sarcoma		1 (2%)			
Leukemia mononuclear	5 (11%)	4 (8%)	4 (8%)	2 (4%)	2 (4%)
Lymphoma malignant				1 (2%)	
Lymph node	(19)	(17)	(22)	(21)	(24)
Leukemia mononuclear				1 (5%)	1 (4%)
Axillary, leukemia mononuclear	1 (5%)	3 (18%)	2 (9%)	2 (10%)	2 (8%)
Axillary, lymphoma malignant				1 (5%)	
Deep cervical, leukemia mononuclear	1 (5%)	1 (6%)	1 (5%)		1 (4%)
Hepatic, leukemia mononuclear			1 (5%)		
Iliac, leukemia mononuclear			1 (5%)		
Inguinal, leukemia mononuclear	1 (5%)				
Lumbar, histiocytic sarcoma		1 (6%)			
Lumbar, leukemia mononuclear	6 (32%)	4 (24%)	5 (23%)	7 (33%)	6 (25%)
Lumbar, lymphoma malignant				1 (5%)	
Mediastinal, histiocytic sarcoma		1 (6%)			
Mediastinal, leukemia mononuclear	6 (32%)	10 (59%)	6 (27%)	12 (57%)	7 (29%)
Pancreatic, leukemia mononuclear	7 (37%)	6 (35%)	8 (36%)	7 (33%)	10 (42%)
Pancreatic, lymphoma malignant				1 (5%)	
Popliteal, leukemia mononuclear	1 (5%)	1 (6%)			
Renal, histiocytic sarcoma		1 (6%)			
Renal, leukemia mononuclear	6 (32%)	3 (18%)	4 (18%)	5 (24%)	8 (33%)
Renal, lymphoma malignant				1 (5%)	
Lymph node, mandibular	(48)	(46)	(48)	(48)	(48)
Adenocarcinoma, metastatic, harderian gland				1 (2%)	
Basal cell carcinoma, metastatic, skin			1 (2%)		
Histiocytic sarcoma		1 (2%)			
Leukemia mononuclear	12 (25%)	10 (22%)	10 (21%)	14 (29%)	12 (25%)
Lymphoma malignant				1 (2%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Hematopoietic System (continued)					
Lymph node, mesenteric	(47)	(47)	(48)	(48)	(48)
Leukemia mononuclear	8 (17%)	6 (13%)	9 (19%)	15 (31%)	8 (17%)
Lymphoma malignant				1 (2%)	
Mesothelioma malignant	1 (2%)				
Spleen	(48)	(48)	(47)	(48)	(48)
Hemangiosarcoma					1 (2%)
Histiocytic sarcoma			1 (2%)		
Leukemia mononuclear	31 (65%)	22 (46%)	23 (49%)	32 (67%)	28 (58%)
Lymphoma malignant				1 (2%)	
Mesothelioma malignant	1 (2%)				
Sarcoma	1 (2%)				
Thymus	(45)	(47)	(46)	(48)	(47)
Histiocytic sarcoma				1 (2%)	
Leukemia mononuclear	3 (7%)	5 (11%)	4 (9%)	10 (21%)	3 (6%)
Lymphoma malignant		1 (2%)		1 (2%)	
Integumentary System					
Mammary gland	(44)	(44)	(43)	(43)	(44)
Fibroadenoma	2 (5%)	5 (11%)	1 (2%)	1 (2%)	3 (7%)
Leukemia mononuclear	1 (2%)	1 (2%)	1 (2%)		
Skin	(48)	(48)	(48)	(48)	(48)
Basal cell carcinoma			2 (4%)	3 (6%)	2 (4%)
Keratoacanthoma	1 (2%)	1 (2%)	4 (8%)	2 (4%)	1 (2%)
Papilloma			1 (2%)		
Squamous cell carcinoma		1 (2%)		1 (2%)	1 (2%)
Squamous cell papilloma				1 (2%)	1 (2%)
Ear, neural crest tumor, benign	1 (2%)				
Ear, neural crest tumor, malignant		2 (4%)			
Sebaceous gland, adenoma	1 (2%)	2 (4%)	2 (4%)		
Subcutaneous tissue, fibroma	3 (6%)	5 (10%)	5 (10%)	3 (6%)	1 (2%)
Subcutaneous tissue, hemangiosarcoma				1 (2%)	1 (2%)
Subcutaneous tissue, lipoma	1 (2%)		1 (2%)		1 (2%)
Subcutaneous tissue, osteosarcoma		1 (2%)			
Subcutaneous tissue, sarcoma			2 (4%)		
Subcutaneous tissue, schwannoma malignant			1 (2%)		
Musculoskeletal System					
Bone	(1)	(1)	(0)	(1)	(1)
Bone, femur	(48)	(48)	(48)	(48)	(48)
Skeletal muscle	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear			1 (2%)		1 (2%)
Mesothelioma malignant	1 (2%)				
Nervous System					
Brain, brain stem	(48)	(48)	(48)	(48)	(48)
Astrocytoma NOS	1 (2%)				
Carcinoma, metastatic, uncertain primary site				1 (2%)	
Leukemia mononuclear	2 (4%)		3 (6%)	2 (4%)	
Brain, cerebellum	(48)	(48)	(48)	(48)	(48)
Astrocytoma NOS					2 (4%)
Leukemia mononuclear	2 (4%)	2 (4%)	3 (6%)	3 (6%)	1 (2%)
Osteosarcoma, metastatic, skin		1 (2%)			

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Nervous System (continued)					
Brain, cerebrum	(48)	(48)	(48)	(48)	(48)
Glioma NOS			1 (2%)		
Leukemia mononuclear	1 (2%)	2 (4%)	2 (4%)		1 (2%)
Peripheral nerve, sciatic	(48)	(48)	(48)	(48)	(48)
Spinal cord, cervical	(47)	(48)	(47)	(48)	(48)
Leukemia mononuclear	1 (2%)		3 (6%)	1 (2%)	3 (6%)
Spinal cord, lumbar	(47)	(48)	(47)	(48)	(48)
Leukemia mononuclear	2 (4%)		3 (6%)	1 (2%)	2 (4%)
Spinal cord, thoracic	(47)	(48)	(47)	(48)	(48)
Leukemia mononuclear	1 (2%)		3 (6%)		1 (2%)
Respiratory System					
Lung	(48)	(48)	(48)	(48)	(48)
Alveolar/bronchiolar adenoma	1 (2%)		1 (2%)	1 (2%)	
Carcinoma, metastatic, intestine small, jejunum					1 (2%)
Carcinoma, metastatic, preputial gland	1 (2%)				
Hepatocellular carcinoma, metastatic, liver			1 (2%)		
Histiocytic sarcoma		1 (2%)	1 (2%)	1 (2%)	
Leukemia mononuclear	16 (33%)	17 (35%)	15 (31%)	23 (48%)	21 (44%)
Lymphoma malignant				1 (2%)	
Neural crest tumor, malignant, metastatic, skin		1 (2%)			
Nose	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear	1 (2%)			1 (2%)	2 (4%)
Trachea	(48)	(48)	(48)	(48)	(48)
Special Senses System					
Eye	(44)	(47)	(47)	(46)	(45)
Harderian gland	(48)	(48)	(48)	(48)	(48)
Adenocarcinoma				1 (2%)	
Leukemia mononuclear		1 (2%)	1 (2%)		
Zymbal's gland	(1)	(2)	(0)	(1)	(1)
Adenoma		1 (50%)			
Carcinoma		1 (50%)		1 (100%)	1 (100%)
Squamous cell carcinoma	1 (100%)				
Urinary System					
Kidney	(47)	(48)	(48)	(48)	(48)
Leukemia mononuclear	3 (6%)	3 (6%)	3 (6%)	2 (4%)	2 (4%)
Lymphoma malignant				1 (2%)	
Mesothelioma malignant	1 (2%)				
Renal tubule, carcinoma					1 (2%)
Transitional epithelium, carcinoma	1 (2%)				
Urethra	(0)	(0)	(1)	(0)	(1)
Bulbourethral gland, leukemia mononuclear					1 (100%)
Urinary bladder	(46)	(48)	(48)	(48)	(48)
Leukemia mononuclear		3 (6%)	1 (2%)	2 (4%)	1 (2%)
Lymphoma malignant				1 (2%)	
Mesothelioma malignant	1 (2%)	1 (2%)			
Systemic Lesions					
Multiple organs	(48)[b]	(48)[b]	(48)[b]	(48)[b]	(48)[b]
Histiocytic sarcoma		1 (2%)	1 (2%)	1 (2%)	
Leukemia mononuclear	31 (65%)	22 (46%)	23 (48%)	32 (67%)	28 (58%)
Lymphoma malignant		1 (2%)		1 (2%)	
Mesothelioma malignant	2 (4%)	2 (4%)	1 (2%)	5 (10%)	8 (17%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Neoplasm Summary					
Total animals with primary neoplasms[c]	46	46	47	48	47
Total primary neoplasms	132	132	150	168	155
Total animals with benign neoplasms	44	43	43	46	46
Total benign neoplasms	81	89	99	99	90
Total animals with malignant neoplasms	38	35	35	44	41
Total malignant neoplasms	50	43	50	69	63
Total animals with metastatic neoplasms	1	2	3	2	1
Total metastatic neoplasms	1	2	3	2	2
Total animals with malignant neoplasms uncertain primary site				1	
Total animals with neoplasms uncertain-benign or malignant	1		1		2
Total uncertain neoplasms	1		1		2

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

TABLE A2
Statistical Analysis of Neoplasms in Male Rats
in the 2-Year Drinking Water Study of Acrylamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Adrenal Medulla: Pheochromocytoma Benign					
Overall rate[a]	8/48 (17%)	4/48 (8%)	5/47 (11%)	10/48 (21%)	1/47 (2%)
Adjusted rate[b]	21.4%	11.3%	13.5%	26.2%	3.1%
Terminal rate[c]	2/17 (12%)	1/14 (7%)	4/19 (21%)	6/16 (38%)	0/9 (0%)
First incidence (days)[d]	516	535	712	670	576
Poly-3 test[e]	P=0.117N	P=0.199N	P=0.276N	P=0.411	P=0.025N
Adrenal Medulla: Pheochromocytoma Malignant					
Overall rate	0/48 (0%)	3/48 (6%)	6/47 (13%)	2/48 (4%)	3/47 (6%)
Adjusted rate	0%	8.7%	16.0%	5.3%	9.3%
Terminal rate	0/17 (0%)	1/14 (7%)	4/19 (21%)	1/16 (6%)	0/9 (0%)
First incidence (days)	-	621	537	684	571
Poly-3 test	P=0.341	P=0.110	P=0.017	P=0.248	P=0.100
Adrenal Medulla: Pheochromocytoma Benign or Malignant					
Overall rate	8/48 (17%)	7/48 (15%)	11/47 (24%)	12/48 (25%)	4/47 (9%)
Adjusted rate	21.4%	19.6%	29.2%	31.3%	12.1%
Terminal rate	2/17 (12%)	2/14 (14%)	8/19 (42%)	7/16 (44%)	0/9 (0%)
First incidence (days)	516	535	537	670	571
Poly-3 test	P=0.264N	P=0.540N	P=0.302	P=0.232	=0.236N
Brain (Brain Stem or Cerebellum): Astrocytoma NOS					
Overall rate	1/48 (2%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	2/48 (4%)
Adjusted rate	2.8%	0.0%	0%	0%	6.0%
Terminal rate	1/17 (6%)	0/14 (0%)	0/19 (0%)	0/16 (0%)	0/9 (0%)
First incidence (days)	737 (T)	-	-	-	576
Poly-3 test	P=0.150	P=0.510N	P=0.492N	P=0.490N	P=0.473
Brain (Cerebrum): Glioma NOS					
Overall rate	0/48 (0%)	0/48 (0%)	1/48 (2%)	0/48 (0%)	0/48 (0%)
Adjusted rate	0%	0%	2.7%	0%	0%
Terminal rate	0/17 (0%)	0/14 (0%)	0/19 (0%)	0/16 (0%)	0/9 (0%)
First incidence (days)	-	-	719	-	-
Poly-3 test	P=0.623N	-	P=0.509	-	-
Epididymis: Malignant Mesothelioma					
Overall rate[a]	2/48 (4%)	2/48 (4%)	1/48 (2%)	5/48 (10)%	8/48 (17%)
Adjusted rate[b]	5.5%	5.8%	2.7%	13.1%	22.9%
Terminal rate[c]	1/17 (6%)	0/14 (0%)	0/19 (19%)	3/16 (19%)	1/9 (11%)
First incidence (days)[d]	533	557	690	620	500
Poly-3 test[e]	P=0.002	P=0.679	P=0.489N	P=0.232	P=0.034
Testes: Interstitial Cell Adenoma					
Overall rate	36/48 (75%)	32/48 (67%)	36/48 (75%)	37/48 (77%)	33/48 (69%)
Adjusted rate	84.2%	80.7%	83.6%	84.6%	80.2%
Terminal rate	15/17 (88%)	13/14 (93%)	16/19 (84%)	14/16 (88%)	7/9 (78%)
First incidence (days)	485	509	537	473	500
Poly-3 test	P=0.406N	P=0.443N	P=0.597N	P=0.609	P=0.410N
Testes: Malignant Mesothelioma					
Overall rate	1/48 (2%)	2/48 (4%)	1/48 (2%)	1/48 (2)%	5/48 (10%)
Adjusted rate	2.7%	5.8%	2.7%	2.7%	14.5%
Terminal rate	0/17 (0%)	0/14 (0%)	0/19 (0%)	0/16 (0%)	0/9 (0%)
First incidence (days)	533	557	690	691	500
Poly-3 test	P=0.030	P=0.484	P=0.755N	P=0.753N	P=0.085

TABLE A2
Statistical Analysis of Neoplasms in Male Rats
in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Epididymis or Testes: Malignant Mesothelioma					
Overall rate	2/48 (4%)	2/48 (4%)	1/48 (2%)	5/48 (10)%	8/48 (17%)
Adjusted rate	5.5%	5.8%	2.7%	13.1%	22.9%
Terminal rate	1/17 (6%)	0/14 (0%)	0/19 (0%)	3/16 (19%)	1/9 (11%)
First incidence (days)	533	557	690	620	500
Poly-3 test	P=0.002	P=0.679	P=0.489N	P=0.232	P=0.034
Heart: Malignant Schwannoma					
Overall rate	1/48 (2%)	2/48 (4%)	3/48 (6%)	4/48 (8%)	6/48 (13%)
Adjusted rate	2.8%	5.9%	7.9%	10.3%	18.3%
Terminal rate	1/17 (6%)	2/14 (14%)	1/19 (5%)	1/16 (6%)	3/9 (33%)
First incidence (days)	737 (T)	737 (T)	537	495	556
Poly-3 test	P=0.015	P=0.483	P=0.328	P=0.201	P=0.040
Liver: Hepatocellular Adenoma					
Overall rate	2/48 (4%)	0/48 (0%)	4/48 (8%)	2/48 (4%)	1/48 (2%)
Adjusted rate	5.6%	0%	10.7%	5.3%	3.1%
Terminal rate	1/17 (6%)	0/14 (0%)	2/19 (11%)	2/16 (13%)	0/9 (0%)
First incidence (days)	697	-	690	737 (T)	647
Poly-3 test	P=0.475N	P=0.248N	P=0.356	P=0.680N	P=0.536N
Liver: Hepatocellular Adenoma or Carcinoma					
Overall rate	2/48 (4%)	0/48 (0%)	5/48 (11%)	2/48 (4%)	1/48 (2%)
Adjusted rate	5.6%	0%	13.1%	5.3%	3.1%
Terminal rate	1/17 (6%)	0/14 (0%)	2/19 (11%)	2/16 (13%)	0/9 (0%)
First incidence (days)	697	-	548	737 (T)	647
Poly-3 test	P=0.432N	P=0.248N	P=0.238	P=0.680N	P=0.536N
Mammary Gland: Fibroadenoma					
Overall rate	2/44 (5%)	5/44 (11%)	1/43 (2%)	1/43 (2%)	3/44 (7%)
Adjusted rate	6.0%	15.5%	2.9%	3.0%	9.9%
Terminal rate	2/16 (13%)	4/14 (29%)	0/19 (0%)	1/15 (7%)	2/9 (22%)
First incidence (days)	737 (T)	688	737	737 (T)	577
Poly-3 test	P=0.569N	P=0.198	P=0.484N	P=0.493N	P=0.458
Oral Mucosa: Squamous Cell Carcinoma					
Overall rate	0/48 (0%)	0/48 (0%)	1/48 (2%)	1/48 (2%)	0/48 (0%)
Adjusted rate	0%	0%	2.7%	2.7%	0%
Terminal rate	0/17 (0%)	0/14 (0%)	1/19 (5%)	0/16 (0%)	0/9 (0%)
First incidence (days)	-	-	737 (T)	717	-
Poly-3 test	P=0.643	-	P=0.508	P=0.510	-
Oral Mucosa: Squamous Cell Papilloma					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	3/48 (6%)	1/48 (2%)
Adjusted rate	0%	0%	0%	8.0%	3.1%
Terminal rate	0/17 (0%)	0/14 (0%)	0/19 (0%)	1/16 (6%)	1/9 (11%)
First incidence (days)	-	-	-	711	737 (T)
Poly-3 test	P=0.102	-	-	P=0.127	P=0.478
Tongue: Squamous Cell Carcinoma					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)
Adjusted rate	0%	0%	0%	0%	3%
Terminal rate	0/17 (0%)	0/14 (0%)	0/19 (0%)	0/16 (0%)	0/9 (0%)
First incidence (days)	-	-	-	-	709
Poly-3 test	P=0.134	-	-	-	P=0.479

TABLE A2
Statistical Analysis of Neoplasms in Male Rats
in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Tongue: Squamous Cell Papilloma					
Overall rate	1/48 (2%)	1/48 (2%)	0/48 (0%)	1/48 (2%)	0/48 (0%)
Adjusted rate	2.8%	2.9%	0%	2.7%	0%
Terminal rate	0/17 (0%)	0/14 (0%)	0/19 (0%)	1/16 (6%)	0/9 (0%)
First incidence (days)	691	701	-	737 (T)	-
Poly-3 test	P=0.357N	P=0.751	P=0.492N	P=0.752N	P=0.523N
Oral Mucosa or Tongue: Squamous Cell Carcinoma or Papilloma					
Overall rate	1/48 (2%)	1/48 (2%)	1/48 (2%)	5/48 (10%)	2/48 (4%)
Adjusted rate	2.8%	2.9%	2.7%	13.2%	6.2%
Terminal rate	0/17 (0%)	0/14 (0%)	1/19 (5%)	2/16 (13%)	1/9 (11%)
First incidence (days)	691	701	737 (T)	711	709
Poly-3 test	P=0.155	P=0.751	P=0.753N	P=0.110	P=0.462
Pituitary (Pars Distalis): Adenoma					
Overall rate	21/48 (44%)	31/48 (65%)	24/47 (51%)	31/48 (65%)	28/47 (60%)
Adjusted rate	54.5%	75.7%	59.9%	74.1%	70.8%
Terminal rate	11/17 (65%)	12/14 (86%)	13/18 (72%)	13/16 (81%)	6/9 (67%)
First incidence (days)	539	452	534	529	492
Poly-3 test	P=0.146	P=0.028 *	P=0.394	P=0.040 *	P=0.086
Pancreas: Acinar Cell Adenoma					
Overall rate	0/46 (0%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	2/48 (4%)
Adjusted rate	0%	0%	0%	0%	6.2%
Terminal rate	0/17 (0%)	0/14 (0%)	0/19 (0%)	0/16 (0%)	1/9 (11%)
First incidence (days)	-	-	-	-	719
Poly-3 test	P=0.021	-	-	-	P=0.216
Pancreatic Islets: Adenoma					
Overall rate	1/46 (2%)	2/48 (4%)	4/48 (8%)	1/48 (2%)	6/48 (13%)
Adjusted rate	2.8%	5.8%	10.4%	2.7%	18.0%
Terminal rate	1/17 (6%)	1/14 (7%)	1/19 (5%)	1/16 (6%)	2/9 (22%)
First incidence (days)	737 (T)	599	564	737 (T)	569
Poly-3 test	P=0.034	P=0.493	P=0.203	P=0.747N	P=0.044
Pancreatic Islets: Carcinoma					
Overall rate	0/46 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)	0/48 (0%)
Adjusted rate	0%	0%	0%	2.7%	0%
Terminal rate	0/17 (0%)	0/14 (0%)	0/19 (0%)	1/16 (6%)	0/9 (0%)
First incidence (days)	-	-	-	737 (T)	-
Poly-3 test	P=0.572	-	-	P=0.513	-
Pancreatic Islets: Adenoma or Carcinoma					
Overall rate	1/46 (2%)	2/48 (4%)	4/48 (8%)	2/48 (4%)	6/48 (13%)
Adjusted rate	2.8%	5.8%	10.4%	5.3%	18.0%
Terminal rate	1/17 (6%)	1/14 (7%)	1/19 (5%)	2/16 (13%)	2/9 (22%)
First incidence (days)	737 (T)	599	564	737 (T)	569
Poly-3 test	P=0.030	P=0.493	P=0.203	P=0.522	P=0.044
Preputial Gland: Adenoma					
Overall rate	0/48 (0%)	3/47 (6%)	4/48 (8%)	1/48 (2%)	4/48 (8%)
Adjusted rate	0%	8.9%	10.6%	2.6%	12.1%
Terminal rate	0/17 (0%)	2/14 (14%)	2/19 (11%)	0/16 (0%)	0/9 (0%)
First incidence (days)	-	621	690	579	586
Poly-3 test	P=0.157	P=0.106	P=0.065	P=0.512	P=0.049

TABLE A2
Statistical Analysis of Neoplasms in Male Rats
in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Preputial Gland: Carcinoma					
Overall rate	7/48 (15%)	3/47 (6%)	4/48 (8%)	4/48 (8%)	2/48 (4%)
Adjusted rate	19.3%	8.6%	10.4%	10.6%	6.1%
Terminal rate	3/17 (18%)	0/14 (0%)	1/19 (5%)	3/16 (19%)	0/9 (0%)
First incidence (days)	691	515	537	624	492
Poly-3 test	P=0.126N	P=0.165N	P=0.222N	P=0.230N	P=0.098N
Preputial Gland: Squamous Cell Carcinoma					
Overall rate	4/48 (8%)	1/47 (2%)	1/48 (2%)	5/48 (10%)	0/48 (0%)
Adjusted rate	10.7%	3.0%	2.7%	12.8%	0%
Terminal rate	1/17 (6%)	0/14 (0%)	1/19 (5%)	1/16 (6%)	0/9 (0%)
First incidence (days)	485	719	737 (T)	473	-
Poly-3 test	P=0.231N	P=0.213N	P=0.177N	P=0.526	P=0.080N
Skin: Basal Cell Carcinoma					
Overall rate	0/48 (0%)	0/48 (0%)	2/48 (4%)	3/48 (6%)	2/48 (4%)
Adjusted rate	0%	0%	5.4%	7.8%	6.2%
Terminal rate	0/17 (0%)	0/14 (0%)	2/19 (11%)	0/16 (0%)	0/9 (0%)
First incidence (days)	-	-	737 (T)	428	698
Poly-3 test	P=0.085	-	P=0.246	P=0.132	P=0.214
Skin: Basal or Squamous Cell Carcinoma					
Overall rate	0/48 (0%)	1/48 (2%)	2/48 (4%)	4/48 (8%)	3/48 (6%)
Adjusted rate	0%	2.9%	5.4%	10.4%	9.2%
Terminal rate	0/17 (0%)	0/14 (0%)	2/19 (11%)	1/16 (6%)	0/9 (0%)
First incidence (days)	-	695	737 (T)	428	686
Poly-3 test	P=0.049	P=0.491	P=0.246	P=0.069	P=0.100
Skin: Keratoacanthoma					
Overall rate	1/48 (2%)	1/48 (2%)	4/48 (8%)	2/48 (4%)	1/48 (2%)
Adjusted rate	2.8%	2.9%	10.7%	5.3%	3.1%
Terminal rate	1/17 (6%)	1/14 (7%)	3/19 (16%)	1/16 (6%)	0/9 (0%)
First incidence (days)	737 (T)	737 (T)	711	711	719
Poly-3 test	P=0.533N	P=0.751	P=0.190	P=0.518	P=0.738
Skin: Keratoacanthoma, Papilloma, Squamous Cell Papilloma					
Overall rate	1/48 (2%)	1/48 (2%)	5/48 (10%)	3/48 (6%)	2/48 (4%)
Adjusted rate	2.8%	2.9%	13.3%	8.0%	6.2%
Terminal rate	1/17 (6%)	1/14 (7%)	3/19 (16%)	2/16 (13%)	1/9 (11%)
First incidence (days)	737 (T)	737 (T)	673	711	719
Poly-3 test	P=0.402	P=0.751	P=0.110	P=0.322	P=0.463
Skin: Keratoacanthoma, Papilloma, Squamous Cell Carcinoma or Papilloma					
Overall rate	1/48 (2%)	2/48 (4%)	5/48 (10%)	4/48 (8%)	3/48 (6%)
Adjusted rate	2.8%	5.8%	13.3%	10.6%	9.3%
Terminal rate	1/17 (6%)	1/14 (7%)	3/19 (16%)	3/16 (19%)	1/9 (11%)
First incidence (days)	737 (T)	695	673	711	686
Poly-3 test	P=0.263	P=0.485	P=0.110	P=0.192	P=0.268
Skin: Adenoma, Basal Cell Carcinoma, Papilloma, Squamous Cell Carcinoma					
Overall rate	1/48 (2%)	3/48 (6%)	5/48 (10%)	4/48 (8%)	3/48 (6%)
Adjusted rate	2.8%	8.6%	13.3%	10.4%	9.2%
Terminal rate	1/17 (6%)	1/14 (7%)	4/19 (21%)	1/16 (6%)	0/9 (0%)
First incidence (days)	737 (T)	557	673	428	686
Poly-3 test	P=0.333	P=0.294	P=0.110	P=0.201	P=0.270

TABLE A2
Statistical Analysis of Neoplasms in Male Rats
in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Skin (Subcutaneous Tissue): Fibroma					
Overall rate	3/48 (6%)	5/48 (10%)	5/48 (10%)	3/48 (6%)	1/48 (2%)
Adjusted rate	8.4%	14.6%	13.1%	8.0%	3.1%
Terminal rate	2/17 (12%)	3/14 (21%)	2/19 (11%)	2/16 (13%)	0/9 (0%)
First incidence (days)	698	695	537	717	647
Poly-3 test	P=0.127N	P=0.328	P=0.388	P=0.643N	P=0.341N
Skin (Subcutaneous Tissue): Fibroma or Sarcoma					
Overall rate	3/48 (6%)	5/48 (10%)	7/48 (15%)	3/48 (6%)	1/48 (2%)
Adjusted rate	8.4%	14.6%	17.8%	8.0%	3.1)
Terminal rate	2/17 (12%)	3/14 (21%)	2/19 (11%)	2/16 (13%)	0/9 (0%)
First incidence (days)	698	695	537	717	647
Poly-3 test	P=0.106N	P=0.328	P=0.191	P=0.643N	P=0.341N
Skin: All Morphologies					
Overall rate	6/48 (13%)	10/48 (21%)	16/48 (33%)	9/48 (19%)	6/48 (13%)
Adjusted rate	16.6%	28.6%	40.2%	23.2%	18.1%
Terminal rate	4/17 (24%)	6/14 (43%)	8/19 (42%)	4/16 (25%)	1/9 (11%)
First incidence (days)	691	557	537	428	647
Poly-3 test	P=0.275N	P=0.174	P=0.018	P=0.336	P=0.562
Thyroid Gland: C-Cell Adenoma					
Overall rate	2/47 (4%)	0/48 (0%)	5/47 (11%)	2/48 (4%)	3/48 (6%)
Adjusted rate	5.6%	0%	13.4%	5.2%	9.3%
Terminal rate	1/17 (6%)	0/14 (0%)	4/19 (21%)	0/16 (0%)	2/9 (22%)
First incidence (days)	670	-	726	585	719
Poly-3 test	P=0.306	P=0.248N	P=0.228	P=0.673N	P=0.450
Thyroid Gland: C-Cell Carcinoma					
Overall rate	0/47 (0%)	1/48 (2%)	0/47 (0%)	0/48 (0%)	1/48 (2%)
Adjusted rate	0.0%	2.9%	0%	0%	3.1%
Terminal rate	0/17 (0%)	0/14 (0%)	0/19 (0%)	0/16 (0%)	0/9 (0%)
First incidence (days)	-	701	-	-	656
Poly-3 test	P=0.377	P=0.492	-	-	P=0.481
Thyroid Gland: Follicular Cell Adenoma					
Overall rate	0/47 (0%)	1/48 (2%)	1/47 (2%)	1/48 (2%)	3/48 (6%)
Adjusted rate	0%	2.9%	2.7%	2.7%	9.2%
Terminal rate	0/17 (0%)	0/14 (0%)	0/19 (0%)	0/16 (0%)	1/9 (11%)
First incidence (days)	-	688	690	711	556
Poly-3 test	P=0.047	P=0.492	P=0.510	P=0.511	P=0.102
Thyroid Gland: Follicular Cell Carcinoma					
Overall rate	1/47 (2%)	2/48 (4%)	3/47 (6%)	6/48 (13%)	6/48 (13%)
Adjusted rate	2.8%	5.8%	7.9%	15.8%	17.6%
Terminal rate	1/17 (6%)	0/14 (0%)	2/19 (11%)	3/16 (19%)	0/9 (0%)
First incidence (days)	737 (T)	630	537	679	569
Poly-3 test	P=0.013	P=0.489	P=0.326	P=0.063	P=0.045
Thyroid Gland: Follicular Cell Adenoma or Carcinoma					
Overall rate	1/47 (2%)	3/48 (6%)	4/47 (9%)	6/48 (13%)	9/48 (19%)
Adjusted rate	2.8%	8.7%	10.5%	15.8%	25.9%
Terminal rate	1/17 (6%)	0/14 (0%)	2/19 (11%)	3/16 (19%)	1/9 (11%)
First incidence (days)	737 (T)	630	537	679	556
Poly-3 test	P=0.002	P=0.294	P=0.196	P=0.063	P=0.005

TABLE A2
Statistical Analysis of Neoplasms in Male Rats
in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Thyroid Gland: C-Cell or Follicular Cell Adenoma or Carcinoma					
Overall rate	3/47 (6%)	4/48 (8%)	8/47 (17%)	8/48 (17%)	12/48 (25%)
Adjusted rate	8.4%	11.5%	21.0%	20.8%	34.3%
Terminal rate	2/17 (12%)	0/14 (0%)	5/19 (26%)	3/16 (19%)	3/9 (33%)
First incidence (days)	670	630	537	585	556
Poly-3 test	P=0.002	P=0.484	P=0.111	P=0.116	P=0.006
All Organs: Hemangiosarcoma or Hemangioma					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)	2/48 (4%)
Adjusted rate	0%	0%	0%	2.7%	6.1%
Terminal rate	0/17 (0%)	0/14 (0%)	0/19 (0%)	1/16 (6%)	0/9 (0%)
First incidence (days)	-	-	-	737 (T)	500
Poly-3 test	P=0.027	-	-	P=0.510	P=0.217
All Organs: Histiocytic Sarcoma					
Overall rate	0/48 (0%)	1/48 (2%)	1/48 (2%)	1/48 (2%)	0/48 (0%)
Adjusted rate	0%	2.9%	2.7%	2.6%	0%
Terminal rate	0/17 (0%)	1/14 (7%)	1/19 (5%)	0/16 (0%)	0/9 (0%)
First incidence (days)	-	737 (T)	737 (T)	647	-
Poly-3 test	P=0.525N	P=0.490	P=0.508	P=0.511	-
All Organs: Leukemia					
Overall rate	31/48 (65%)	22/48 (46%)	23/48 (48%)	32/48 (67%)	28/48 (58%)
Adjusted rate	75.1%	55.6%	55.7%	73.3%	68.7%
Terminal rate	13/17 (77%)	4/14 (29%)	10/19 (53%)	12/16 (75%)	5/9 (56%)
First incidence (days)	396	509	508	495	410
Poly-3 test	P=0.329	P=0.041N	P=0.039N	P=0.524N	P=0.333N
All Organs: Malignant Lymphoma					
Overall rate	0/48 (0%)	1/48 (2%)	0/48 (0%)	1/48 (2%)	0/48 (0%)
Adjusted rate	0%	2.9%	0%	2.7%	0%
Terminal rate	0/17 (0%)	0/14 (0%)	0/19 (0%)	0/16 (0%)	0/9 (0%)
First incidence (days)	-	239	-	691	-
Poly-3 test	P=0.621N	P=0.496	-	P=0.511	-
All Organs: Mesothelioma					
Overall rate	2/48 (4%)	2/48 (4%)	1/48 (2%)	5/48 (10%)	8/48 (17%)
Adjusted rate	5.5%	5.8%	2.7%	13.1%	22.9%
Terminal rate	1/17 (6%)	0/14 (0%)	0/19 (0%)	3/16 (19%)	1/9 (11%)
First incidence (days)	533	557	690	620	500
Poly-3 test	P=0.002	P=0.679	P=0.489N	P=0.232	P=0.034
All Organs: Osteosarcoma or Osteoma					
Overall rate	0/48 (0%)	1/48 (2%)	0/48 (0%)	0/48 (0%)	0/48 (0%)
Adjusted rate	0%	2.9%	0%	0%	0%
Terminal rate	0/17 (0%)	1/14 (7%)	0/19 (0%)	0/16 (0%)	0/9 (0%)
First incidence (days)	-	737 (T)	-	-	-
Poly-3 test	P=0.493N	P=0.490	-	-	-
All Organs: Benign Neoplasms					
Overall rate	44/48 (92%)	43/48 (90%)	43/48 (90%)	46/48 (96%)	46/48 (96%)
Adjusted rate	97.4%	97.3%	95.3%	98.7%	99.2%
Terminal rate	17/17 (100%)	14/14 (100%)	19/19 (100%)	16/16 (100%)	9/9 (100%)
First incidence (days)	485	452	534	473	492
Poly-3 test	P=0.192	P=0.820N	P=0.516N	P=0.690	P=0.586

TABLE A2
Statistical Analysis of Neoplasms in Male Rats
in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
All Organs: Malignant Neoplasms					
Overall rate	38/48 (79%)	35/48 (73%)	35/48 (73%)	44/48 (92%)	41/48 (85%)
Adjusted rate	85.3%	79.9%	78.3%	93.0%	91.0%
Terminal rate	13/17 (77%)	8/14 (57%)	14/19 (74%)	15/16 (94%)	7/9 (78%)
First incidence (days)	396	239	508	428	410
Poly-3 test	P=0.051	P=0.338N	P=0.264N	P=0.177	P=0.290
All Organs: Benign or Malignant Neoplasms					
Overall rate	46/48 (96%)	46/48 (96%)	47/48 (98%)	48/48 (100%)	47/48 (98%)
Adjusted rate	98.8%	99.2%	99.7%	100.0%	99.6%
Terminal rate	17/17 (100%)	14/14 (100%)	19/19 (100%)	16/16 (100%)	9/9 (100%)
First incidence (days)	396	239	508	428	410
Poly-3 test	P=0.570	P=0.928	P=0.874	P=0.819	P=0.887

[a] Number of animals with neoplasm per number of animals examined microscopically.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[c] Observed incidence at the terminal sacrifice.
[d] T indicates terminal sacrifice.
[e] Beneath the 0 mM acrylamide are the p values associated with the trend test. Beneath the treated (0.0875, 0.175, 0.35, and 0.70 mM acrylamide) group incidences are the p values corresponding to pairwise comparisons between the 0 mM acrylamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice. An N indicates a negative trend or decreased tumor incidence.

TABLE A3a
Historical Incidence of Thyroid Gland Follicular Cell Neoplasms in NCTR Control Male F344/N Rats

Study (Report Date)	Route of Administration	Incidence in Controls	
		Carcinoma	Adenoma or Carcinoma
Doxylamine (April 1991)	Diet	0/48 (0.0%)	1/48 (2.1%)
Fumonisin B_1 (March 1999)	Diet	0/48 (0.0%)	0/48 (0.0%)
Gentian Violet (November 1988)	Diet	0/163 (0.0%)	1/163 (0.6%)
Leucomalachite Green (June 2001)	Diet	0/47 (0.0%)	0/47 (0.0%)
Pyrilamine (July 1991)	Diet	0/42 (0.0%)	0/42 (0.0%)
Sulfamethazine (February 1988)	Diet	0/170 (0.0%)	0/170 (0.0%)
Triprolidine (June 1991)	Diet	0/40 (0.0%)	0/40 (0.0%)
Total (%)		0/558 (0.0%)	2/558 (0.4%)
Range		0.0%	0.0%-2.1%

TABLE A3b
Historical Incidence of Mesothelioma (All Sites) in NCTR Control Male F344/N Rats

Study (Report Date)	Route of Administration	Incidence in Controls
Doxylamine (April 1991)	Diet	2/48 (4.2%)
Fumonisin B_1 (March 1999)	Diet	3/48 (6.3%)
Gentian Violet (November 1988)	Diet	6/180 (3.3%)
Leucomalachite Green (June 2001)	Diet	2/48 (4.2%)
Pyrilamine (July 1991)	Diet	2/48 (4.2%)
Sulfamethazine (February 1988)	Diet	11/179 (6.1%)
Triprolidine (June 1991)	Diet	3/47 (6.4%)
Total (%)		29/598 (4.8%)
Range		3.3%-6.4%

TABLE A3c
Historical Incidence of Malignant Schwannoma of the Heart in NCTR Control Male F344/N Rats

Study (Report Date)	Route of Administration	Incidence in Controls
Doxylamine (April 1991)	Diet	0/48 (0.0%)
Fumonisin B_1 (March 1999)	Diet	0/48 (0.0%)
Gentian Violet (November 1988)	Diet	0/180 (0.0%)
Leucomalachite Green (June 2001)	Diet	0/48 (0.0%)
Pyrilamine (July 1991)	Diet	0/48 (0.0%)
Sulfamethazine (February 1988)	Diet	0/179 (0.0%)
Triprolidine (June 1991)	Diet	0/47(0.0%)
Total (%)		0/598 (0.0%)
Range		0.0%

TABLE A3d
Historical Incidence of Adenoma of the Pancreatic Islets in NCTR Control Male F344/N Rats

Study (Report Date)	Route of Administration	Incidence in Controls
Doxylamine (April 1991)	Diet	2/48 (4.2%)
Fumonisin B_1 (March 1999)	Diet	4/47 (8.5%)
Gentian Violet (November 1988)	Diet	22/168 (13.1%)
Leucomalachite Green (June 2001)	Diet	5/48 (10.4%)
Pyrilamine (July 1991)	Diet	3/46 (6.5%)
Sulfamethazine (February 1988)	Diet	15/177 (8.5%)
Triprolidine (June 1991)	Diet	2/47 (4.3%)
Total (%)		53/581 (8.8%)
Range		4.2%-13.1%

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Acrylamide[a]

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Disposition Summary					
Animals initially in study	48	48	48	48	48
Early deaths					
Moribund sacrifice	18	20	20	23	27
Natural deaths	6	4	2		4
Survivors					
Moribund sacrifice	5	9	7	7	7
Natural deaths	2	1		2	1
Terminal sacrifice	17	14	19	16	9
Animals examined microscopically	48	48	48	48	48
Alimentary System					
Intestine large, cecum	(45)	(48)	(47)	(48)	(47)
Dilatation				1 (2%)	
Inflammation					1 (2%)
Ulcer					1 (2%)
Lymphoid tissue, hyperplasia				3 (6%)	
Intestine large, colon	(45)	(47)	(47)	(48)	(48)
Lymphoid tissue, hyperplasia			2 (4%)	1 (2%)	
Intestine small, duodenum	(45)	(48)	(47)	(48)	(48)
Mucosa, hyperplasia				1 (2%)	
Intestine small, ileum	(44)	(47)	(47)	(48)	(47)
Lymphoid tissue, hyperplasia	2 (5%)	1 (2%)	2 (4%)	3 (6%)	
Intestine small, jejunum	(43)	(46)	(46)	(48)	(45)
Inflammation			1 (2%)		1 (2%)
Ulcer					1 (2%)
Lymphoid tissue, hyperplasia			1 (2%)	2 (4%)	
Liver	(48)	(48)	(48)	(48)	(48)
Angiectasis	3 (6%)		1 (2%)	1 (2%)	
Basophilic focus	1 (2%)				
Basophilic focus, multiple	2 (4%)			1 (2%)	1 (2%)
Cyst			1 (2%)		1 (2%)
Degeneration, cystic	5 (10%)	4 (8%)	7 (15%)	6 (13%)	11 (23%)
Eosinophilic focus	2 (4%)	1 (2%)	1 (2%)	5 (10%)	3 (6%)
Eosinophilic focus, multiple		1 (2%)		1 (2%)	1 (2%)
Granuloma	7 (15%)	5 (10%)	6 (13%)	4 (8%)	4 (8%)
Hematopoietic cell proliferation	1 (2%)	3 (6%)	2 (4%)	1 (2%)	1 (2%)
Hepatodiaphragmatic nodule		1 (2%)	1 (2%)	1 (2%)	2 (4%)
Infiltration cellular, lymphocyte		1 (2%)			1 (2%)
Necrosis, coagulative			1 (2%)		2 (4%)
Pigmentation				1 (2%)	
Regeneration				1 (2%)	
Thrombosis				1 (2%)	
Vacuolization cytoplasmic	11 (23%)	6 (13%)	10 (21%)	10 (21%)	2 (4%)
Bile duct, hyperplasia	16 (33%)	19 (40%)	21 (44%)	17 (35%)	21 (44%)
Caudate lobe, developmental malformation	1 (2%)		1 (2%)		
Centrilobular, degeneration			1 (2%)		
Centrilobular, necrosis	3 (6%)	1 (2%)		2 (4%)	1 (2%)
Hepatocyte, hyperplasia	1 (2%)	2 (4%)			1 (2%)
Left lateral lobe, developmental malformation	3 (6%)		2 (4%)	2 (4%)	1 (2%)
Median lobe, developmental malformation				1 (2%)	
Oval cell, hyperplasia		1 (2%)		1 (2%)	
Right lateral lobe, developmental malformation	2 (4%)			2 (4%)	1 (2%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Alimentary System (continued)					
Mesentery	(2)	(4)	(7)	(7)	(4)
Accessory spleen	1 (50%)			1 (14%)	
Polyarteritis			1 (14%)		
Fat, necrosis		3 (75%)	7 (100%)	5 (71%)	3 (75%)
Oral Mucosa	(0)	(0)	(2)	(6)	(3)
Hyperplasia				2 (33%)	2 (67%)
Pancreas	(46)	(48)	(48)	(48)	(48)
Accessory spleen			1 (2%)		
Infiltration cellular, lymphocyte		1 (2%)			
Inflammation					1 (2%)
Polyarteritis	2 (4%)	3 (6%)	1 (2%)	1 (2%)	1 (2%)
Acinar cell, atrophy	17 (37%)	15 (31%)	15 (31%)	14 (29%)	9 (19%)
Acinar cell, hyperplasia			1 (2%)		
Mesothelium, hyperplasia					1 (2%)
Salivary glands	(48)	(48)	(48)	(48)	(48)
Infiltration cellular, plasma cell	1 (2%)				
Stomach, Forestomach	(47)	(48)	(47)	(48)	(48)
Edema	3 (6%)	3 (6%)	3 (6%)	1 (2%)	2 (4%)
Hyperplasia	2 (4%)	6 (13%)	4 (9%)	2 (4%)	4 (8%)
Inflammation		3 (6%)	2 (4%)	4 (8%)	1 (2%)
Ulcer		3 (6%)	4 (9%)	4 (8%)	
Stomach, glandular	(47)	(48)	(47)	(48)	(48)
Amyloid deposition				1 (2%)	
Edema	1 (2%)	2 (4%)			
Erosion		1 (2%)			
Hemorrhage				1 (2%)	
Inflammation		2 (4%)	2 (4%)	1 (2%)	
Ulcer		1 (2%)			
Tongue	(3)	(1)	(0)	(2)	(1)
Hyperplasia	1 (33%)				
Cardiovascular System					
Blood vessel	(48)	(48)	(48)	(48)	(48)
Heart	(48)	(48)	(48)	(48)	(48)
Cardiomyopathy	37 (77%)	37 (77%)	36 (75%)	38 (79%)	38 (79%)
Dilatation			1 (2%)	1 (2%)	
Infarct	1 (2%)				
Inflammation					1 (2%)
Atrium, thrombosis	13 (27%)	13 (27%)	8 (17%)	11 (23%)	10 (21%)
Myocardium, hypertrophy				1 (2%)	
Endocrine System					
Adrenal cortex	(48)	(48)	(48)	(48)	(48)
Accessory adrenal cortical nodule					1 (2%)
Angiectasis	5 (10%)	3 (6%)	5 (10%)	1 (2%)	3 (6%)
Atrophy	1 (2%)				2 (4%)
Hyperplasia, focal	5 (10%)	2 (4%)		2 (4%)	
Hypertrophy, focal	4 (8%)	3 (6%)	8 (17%)	6 (13%)	8 (17%)
Necrosis, coagulative		1 (2%)			
Thrombus				1 (2%)	
Vacuolization cytoplasmic	12 (25%)	16 (33%)	12 (25%)	11 (23%)	13 (27%)
Adrenal Medulla	(48)	(48)	(47)	(48)	(47)
Angiectasis	1 (2%)	3 (6%)	4 (9%)	2 (4%)	2 (4%)
Hyperplasia, focal	6 (13%)	7 (15%)	4 (9%)	2 (4%)	5 (11%)
Islets, pancreatic	(46)	(48)	(48)	(48)	(48)
Hyperplasia				1 (2%)	

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Endocrine System (continued)					
Parathyroid gland	(46)	(48)	(47)	(47)	(44)
Atrophy					1 (2%)
Cyst		1 (2%)			
Hyperplasia, focal	1 (2%)				
Pituitary gland	(48)	(48)	(47)	(48)	(47)
Angiectasis	2 (4%)		1 (2%)	1 (2%)	2 (4%)
Pigmentation				1 (2%	
Thrombosis			1 (2%)		
Pars distalis, cyst	6 (13%)		3 (6%)		2 (4%)
Pars distalis, hyperplasia	5 (10%)	1 (2%)	2 (4%)	2 (4%)	3 (6%)
Pars intermedia, cyst		1 (2%)		2 (4%)	
Thyroid gland	(47)	(48)	(47)	(48)	(48)
C-cell, hyperplasia	3 (6%)	4 (8%)	2 (4%)	4 (8%)	5 (10%)
Follicle, cyst	1 (2%)	2 (4%)	3 (6%)	2 (4%)	2 (4%)
Follicular cell, hyperplasia		2 (4%)			3 (6%)
General Body System					
Peritoneum	(0)	(0)	(0)	(0)	(2)
Tissue NOS	(0)	(1)	(1)	(1)	(1)
Fat, necrosis				1 (100%)	
Fat, scrotal, necrosis					1 (100%)
Genital System					
Epididymis	(48)	(48)	(48)	(48)	(48)
Atrophy		1 (2%)	2 (4%)	1 (2%)	
Exfoliated germ cell	24 (50%)	19 (40%)	23 (48%)	26 (54%)	24 (50%)
Granuloma sperm	1 (2%)				
Hypospermia	31 (65%)	32 (67%)	30 (63%)	29 (60%)	24 (50%)
Fat, necrosis		1 (2%)			
Mesothelium, hyperplasia	2 (4%)				
Serosa, cyst			1 (2%)		
Penis	(1)	(0)	(0)	(1)	(1)
Inflammation	1 (100%)				
Preputial gland	(48)	(47)	(48)	(48)	(48)
Atrophy		1 (2%)	1 (2%)		
Inflammation	35 (73%)	38 (81%)	32 (67%)	34 (71%)	35 (73%)
Duct, ectasia	4 (8%)	6 (13%)	11 (23%)	14 (29%)	10 (21%)
Glandular, hyperplasia	1 (2%)		1 (2%)	1 (2%)	
Prostate	(47)	(48)	(48)	(48)	(48)
Atrophy		1 (2%)			
Inflammation	25 (53%)	29 (60%)	28 (58%)	32 (67%)	25 (52%)
Seminal vesicle	(48)	(48)	(47)	(48)	(48)
Atrophy	6 (13%)	12 (25%)	9 (19%)	9 (19%)	9 (19%)
Decreased secretory fluid	3 (6%)	6 (13%)	5 (11%)	1 (2%)	1 (2%)
Lumen, distended			1 (2%)		
Testes	(48)	(48)	(48)	(48)	(48)
Granuloma sperm		1 (2%)			
Inflammation	1 (2%)	1 (2%)			
Polyarteritis	1 (2%)		1 (2%)		1 (2%)
Interstitial cell, hyperplasia	6 (13%)	6 (13%)	2 (4%)	3 (6%)	3 (6%)
Seminiferous tubule, atrophy	13 (27%)	21 (44%)	15 (31%)	13 (27%)	16 (33%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Hematopoietic System					
Bone marrow	(47)	(48)	(48)	(48)	(48)
Atrophy	2 (4%)	4 (8%)	5 (10%)	2 (4%)	4 (8%)
Hyperplasia	3 (6%)	3 (6%)	2 (4%)	1 (2%)	3 (6%)
Myeloid cell, hyperplasia					1 (2%)
Lymph node	(19)	(17)	(22)	(21)	(24)
Degeneration, cystic					1 (4%)
Hyperplasia, lymphoid		1 (6%)			
Inflammation					1 (4%)
Axillary, hemorrhage		1 (6%)			
Axillary, hyperplasia, lymphoid	2 (11%)				
Deep cervical, degeneration, cystic			1 (5%)		
Inguinal, degeneration, cystic	2 (11%)				
Inguinal, hyperplasia, lymphoid	1 (5%)				
Inguinal, infiltration cellular, plasma cell	1 (5%)				
Lumbar, degeneration, cystic	3 (16%)	2 (12%)	2 (9%)		
Lumbar, hyperplasia, lymphoid	1 (5%)				1 (4%)
Lumbar, infiltration cellular, plasma cell	2 (11%)				
Lumbar, medulla sinus, dilatation				1 (5%)	
Mediastinal, degeneration, cystic		1 (6%)	1 (5%)	3 (14%)	
Mediastinal, hemorrhage	1 (5%)	1 (6%)	1 (5%)	1 (5%)	
Mediastinal, hyperplasia, lymphoid	1 (5%)				1 (4%)
Mediastinal, infiltration cellular, mast cell					1 (4%)
Mediastinal, medulla sinus, dilatation				1 (5%)	1 (4%)
Medulla, pancreatic sinus, dilatation		1 (6%)		2 (10%)	
Medulla, renal suinus, dilatation			1 (5%)		1 (4%)
Pancreatic, degeneration, cystic			1 (5%)		
Pancreatic, hemorrhage	1 (5%)				
Pancreatic, hyperplasia, lymphoid	1 (5%)	1 (6%)	1 (5%)		1 (4%)
Renal, degeneration, cystic	3 (16%)	1 (6%)	5 (23%)	4 (19%)	3 (13%)
Renal, hemorrhage				1 (5%)	
Renal, hyperplasia, lymphoid		1 (6%)	2 (9%)		
Lymph node, mandibular	(48)	(46)	(48)	(48)	(48)
Degeneration, cystic	7 (15%)	7 (15%)	11 (23%)	12 (25%)	12 (25%)
Hemorrhage	1 (2%)	1 (2%)	1 (2%)	1 (2%)	
Hyperplasia, lymphoid	5 (10%)	1 (2%)	1 (2%)	6 (13%)	2 (4%)
Infiltration cellular, plasma cell	9 (19%)	10 (22%)	12 (25%)	6 (13%)	18 (38%)
Medulla, sinus dilatation				1 (2%)	
Lymph node, mesenteric	(47)	(47)	(48)	(48)	(48)
Degeneration, cystic	3 (6%)	2 (4%)	2 (4%)	4 (8%)	5 (10%)
Hemorrhage	1 (2%)	1 (2%)		3 (6%)	3 (6%)
Hyperplasia, lymphoid	2 (4%)	2 (4%)	1 (2%)		4 (8%)
Infiltration cellular, plasma cell				1 (2%)	
Medulla, sinus, dilatation	1 (2%)	1 (2%)		1 (2%)	
Spleen	(48)	(48)	(47)	(48)	(48)
Accessory spleen		2 (4%)		4 (8%)	2 (4%)
Congestion			1 (2%)		
Developmental malformation			1 (2%)		
Hematopoietic cell proliferation	3 (6%)	3 (6%)	4 (9%)	1 (2%)	
Hyperplasia, lymphoid		1 (2%)	1 (2%)		
Infarct	4 (8%)	10 (21%)	11 (23%)	10 (21%)	9 (19%)
Pigmentation	3 (6%)	3 (6%)	4 (9%)	3 (6%)	1 (2%)
Capsule, hematocyst				1 (2%)	
Red pulp, atrophy	1 (2%)	1 (2%)	2 (4%)	3 (6%)	4 (8%)
Thymus	(45)	(47)	(46)	(48)	(47)
Atrophy	40 (89%)	41 (87%)	40 (87%)	35 (73%)	43 (91%)
Cyst	1 (2%)			1 (2%)	1 (2%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Integumentary System					
Mammary gland	(44)	(44)	(43)	(43)	(44)
Galactocele	3 (7%)	10 (23%)	7 (16%)	11 (26%)	9 (20%)
Inflammation			1 (2%)		
Lactation	17 (39%)	19 (43%)	19 (44%)	23 (53%)	19 (43%)
Alveolus, hyperplasia	11 (25%)	13 (30%)	12 (28%)	14 (33%)	10 (23%)
Skin	(48)	(48)	(48)	(48)	(48)
Abscess					1 (2%)
Cyst epithelial inclusion		2 (4%)		2 (4%)	1 (2%)
Inflammation		1 (2%)	1 (2%)		
Ulcer		2 (4%)			
Epidermis, hyperplasia			1 (2%)		
Fat, subcutaneous tissue, necrosis	1 (2%)				1 (2%)
Subcutaneous tissue, metaplasia, osseous				1 (2%)	
Tail, hyperkeratosis					1 (2%)
Musculoskeletal System					
Bone	(1)	(1)	(0)	(1)	(1)
Cranium, fracture					1 (100%)
Vertebra, fracture	1 (100%)	1 (100%)		1 (100%)	
Bone, femur	(48)	(48)	(48)	(48)	(48)
Fibrous osteodystrophy	1 (2%)	2 (4%)			
Skeletal muscle	(48)	(48)	(48)	(48)	(48)
Nervous System					
Brain, brain stem	(48)	(48)	(48)	(48)	(48)
Gliosis, focal	1 (2%)				
Hemorrhage		1 (2%)	1 (2%)	1 (2%)	
Hypothalamus, compression	6 (13%)	13 (27%)	13 (27%)	16 (33%)	9 (19%)
Ventricle, dilatation	1 (2%)	1 (2%)	1 (2%)		
Brain, cerebellum	(48)	(48)	(48)	(48)	(48)
Hemorrhage					1 (2%)
Brain, cerebrum	(48)	(48)	(48)	(48)	(48)
Hemorrhage					1 (2%)
Hydrocephalus		2 (4%)	1 (2%)		1 (2%)
Mineralization	1 (2%)				
Necrosis, focal		1 (2%)			
Thrombosis		1 (2%)			
Perivascular, infiltration cellular, mixed cell, focal			1 (2%)		
Ventricle, dilatation	1 (2%)	5 (10%)	6 (13%)	5 (10%)	1 (2%)
Peripheral nerve, sciatic	(48)	(48)	(48)	(48)	(48)
Axon, degeneration	5 (10%)	7 (15%)	7 (15%)	11 (23%)	23 (48%)
Spinal cord, cervical	(47)	(48)	(47)	(48)	(48)
Degeneration, focal					1 (2%)
Hemorrhage			1 (2%)		
Axon, degeneration	20 (43%)	22 (46%)	20 (43%)	22 (46%)	19 (40%)
Nerve, degeneration	2 (4%)	1 (2%)	3 (6%)		
Spinal cord, lumbar	(47)	(48)	(47)	(48)	(48)
Cyst	1 (2%)				
Gliosis, focal		1 (2%)			
Axon, degeneration	6 (13%)	4 (8%)	5 (11%)	1 (2%)	1 (2%)
Nerve, degeneration	16 (34%)	20 (42%)	20 (43%)	16 (33%)	16 (33%)
Spinal cord, thoracic	(47)	(48)	(47)	(48)	(48)
Hemorrhage, focal			1 (2%)		
Axon, degeneration	22 (47%)	18 (38%)	25 (53%)	32 (67%)	21 (44%)
Nerve, degeneration	4 (9%)	1 (2%)	2 (4%)	2 (4%)	

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Respiratory System					
Lung	(48)	(48)	(48)	(48)	(48)
Granuloma	2 (4%)		1 (2%)	1 (2%)	
Hemorrhage					1 (2%)
Inflammation		2 (4%)	2 (4%)	1 (2%)	5 (10%)
Alveolar epithelium, hyperplasia	1 (2%)	2 (4%)	5 (10%)		4 (8%)
Alveolus, infiltration cellular, histiocyte	6 (13%)	4 (8%)	6 (13%)	6 (13%)	4 (8%)
Nose	(48)	(48)	(48)	(48)	(48)
Fungus	1 (2%)	1 (2%)			
Inflammation	2 (4%)	3 (6%)	8 (17%)	6 (13%)	1 (2%)
Keratin cyst	1 (2%)				
Trachea	(48)	(48)	(48)	(48)	(48)
Inflammation, chronic		1 (2%)			
Epithelium, hyperplasia		1 (2%)			
Special Senses System					
Eye	(44)	(47)	(47)	(46)	(45)
Cataract	1 (2%)	1 (2%)			
Phthisis bulbi	1 (2%)			1 (2%)	
Cornea, inflammation		1 (2%)			
Retina, degeneration	2 (5%)	2 (4%)	3 (6%)	2 (4%)	10 (22%)
Sclera, metaplasia, osseous	8 (18%)	3 (6%)	1 (2%)	1 (2%)	4 (9%)
Harderian gland	(48)	(48)	(48)	(48)	(48)
Infiltration cellular, lymphocyte	7 (15%)	8 (17%)	7 (15%)	2 (4%)	9 (19%)
Zymbal's gland	(1)	(2)	(0)	(1)	(1)
Urinary System					
Kidney	(47)	(48)	(48)	(48)	(48)
Accumulation, hyaline droplet				1 (2%)	
Cyst	1 (2%)	1 (2%)	1 (2%)		
Hydronephrosis	1 (2%)	2 (4%)		2 (4%)	
Infarct		1 (2%)			
Nephropathy	46 (98%)	45 (94%)	47 (98%)	48 (100%)	46 (96%)
Pigmentation				1 (2%)	
Cortex, inflammation, chronic		1 (2%)		1 (2%)	
Urethra	(0)	(0)	(1)	(0)	(1)
Bulbourethral gland, dilatation			1 (100%)		
Bulbourethral gland, inflammation					1 (100%)
Urinary bladder	(46)	(48)	(48)	(48)	(48)
Calculus gross observation		1 (2%)			
Dilatation	3 (7%)	3 (6%)	2 (4%)		1 (2%)
Hemorrhage					1 (2%)

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

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF ACRYLAMIDE

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Drinking Water Study of Acrylamide[a]

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Disposition Summary					
Animals initially in study	48	48	48	48	48
Early deaths					
Moribund sacrifice	8	13	17	14	23
Natural deaths	3	2	2	5	2
Survivors					
Moribund sacrifice	2	5	7	6	10
Natural deaths	1		1		
Terminal sacrifice	34	28	21	23	13
Animals examined microscopically	48	48	48	48	48
Alimentary System					
Esophagus	(48)	(48)	(48)	(48)	(48)
Mesothelioma malignant	1 (2%)				
Intestine large, cecum	(47)	(48)	(48)	(46)	(47)
Leukemia mononuclear		1 (2%)	2 (4%)		
Intestine large, colon	(46)	(48)	(48)	(47)	(47)
Intestine small, duodenum	(48)	(48)	(48)	(46)	(47)
Leukemia mononuclear			1 (2%)		
Intestine small, ileum	(47)	(48)	(48)	(46)	(46)
Leukemia mononuclear		1 (2%)	2 (4%)		
Intestine small, jejunum	(46)	(48)	(48)	(45)	(46)
Liver	(48)	(48)	(48)	(48)	(48)
Hepatocellular adenoma			1 (2%)	1 (2%)	3 (6%)
Histiocytic sarcoma		1 (2%)			1 (2%)
Leukemia mononuclear	10 (21%)	15 (31%)	16 (33%)	13 (27%)	14 (29%)
Lymphoma malignant					1 (2%)
Sarcoma stromal, metastatic, uterus		1 (2%)			
Mesentery	(7)	(9)	(10)	(4)	(5)
Leukemia mononuclear	1 (14%)		4 (40%)		1 (20%)
Mesothelioma malignant	1 (14%)				
Oral mucosa	(0)	(2)	(2)	(3)	(7)
Squamous cell carcinoma					1 (14%)
Squamous cell papilloma		2 (100%)	1 (50%)	2 (67%)	4 (57%)
Pancreas	(48)	(48)	(48)	(47)	(48)
Histiocytic sarcoma		1 (2%)			
Leukemia mononuclear	2 (4%)	2 (4%)	3 (6%)	1 (2%)	1 (2%)
Lymphoma malignant				1 (2%)	
Salivary glands	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear	1 (2%)		2 (4%)		1 (2%)
Stomach, forestomach	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear			3 (6%)		
Lymphoma malignant				1 (2%)	
Squamous cell papilloma			2 (4%)		
Stomach, glandular	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear			2 (4%)		
Tongue	(0)	(0)	(0)	(1)	(3)
Squamous cell carcinoma					1 (33%)
Squamous cell papilloma				1 (100%)	1 (33%)
Cardiovascular System					
Heart	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear	1 (2%)	3 (6%)	6 (13%)	2 (4%)	2 (4%)
Schwannoma malignant	2 (4%)	1 (2%)		2 (4%)	4 (8%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats
in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Endocrine System					
Adrenal cortex	(48)	(48)	(48)	(48)	(48)
Adenoma	1 (2%)	1 (2%)		1 (2%)	
Leukemia mononuclear	1 (2%)	2 (4%)	3 (6%)	3 (6%)	4 (8%)
Adrenal medulla	(48)	(48)	(48)	(47)	(48)
Leukemia mononuclear	2 (4%)	2 (4%)	5 (10%)	2 (4%)	6 (13%)
Lymphoma malignant		1 (2%)			
Pheochromocytoma complex					1 (2%)
Pheochromocytoma malignant	2 (4%)	3 (6%)	1 (2%)	1 (2%)	1 (2%)
Bilateral, pheochromocytoma malignant		1 (2%)			1 (2%)
Islets, pancreatic	(48)	(48)	(48)	(47)	(48)
Adenoma	1 (2%)	1 (2%)			
Leukemia mononuclear		1 (2%)	2 (4%)		
Parathyroid gland	(47)	(47)	(45)	(46)	(46)
Adenoma		1 (2%)			
Leukemia mononuclear			1 (2%)		
Pituitary gland	(48)	(48)	(47)	(48)	(48)
Leukemia mononuclear	1 (2%)	1 (2%)	1 (2%)		
Lymphoma malignant				1 (2%)	
Pars distalis, adenoma	37 (77%)	35 (73%)	33 (70%)	29 (60%)	28 (58%)
Pars distalis, carcinoma				1 (2%)	
Thyroid gland	(48)	(48)	(48)	(48)	(47)
Leukemia mononuclear			2 (4%)		
C-cell, adenoma	1 (2%)	4 (8%)	4 (8%)	3 (6%)	4 (9%)
C-cell, carcinoma	1 (2%)		2 (4%)		
Follicular cell, adenoma			1 (2%)		2 (4%)
Follicular cell, carcinoma			1 (2%)	3 (6%)	2 (4%)
General Body System					
Tissue NOS	(0)	(0)	(1)	(0)	(1)
Sarcoma					1 (100%)
Fat, leukemia mononuclear			1 (100%)		
Genital System					
Clitoral gland	(48)	(48)	(47)	(48)	(47)
Adenoma	9 (19%)	7 (15%)	5 (11%)	8 (17%)	3 (6%)
Carcinoma	1 (2%)	5 (10%)	12 (26%)	3 (6%)	8 (17%)
Leukemia mononuclear	1 (2%)		1 (2%)		
Squamous cell carcinoma				1 (2%)	
Squamous cell papilloma			1 (2%)		3 (6%)
Bilateral, carcinoma		1 (2%)			
Ovary	(48)	(48)	(48)	(48)	(48)
Granulosa cell tumor benign	2 (4%)				
Histiocytic sarcoma		1 (2%)			1 (2%)
Leukemia mononuclear	1 (2%)	3 (6%)	2 (4%)	3 (6%)	1 (2%)
Uterus	(48)	(48)	(48)	(48)	(48)
Histiocytic sarcoma		1 (2%)			
Leukemia mononuclear	1 (2%)		1 (2%)		
Polyp stromal	9 (19%)	12 (25%)	8 (17%)	10 (21%)	12 (25%)
Polyp stromal, multiple			1 (2%)		
Sarcoma stromal		4 (8%)	3 (6%)		4 (8%)
Endometrium, adenoma		1 (2%)			
Vagina	(1)	(4)	(1)	(4)	(5)
Sarcoma stromal, metastatic, uterus					1 (20%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Hematopoietic System					
Bone marrow	(48)	(48)	(48)	(47)	(48)
Leukemia mononuclear					1 (2%)
Lymph node	(7)	(9)	(10)	(6)	(9)
Axillary, leukemia mononuclear		3 (33%)	1 (10%)		
Cervical, leukemia mononuclear		1 (11%)			
Deep cervical, leukemia mononuclear		1 (11%)		1 (17%)	1 (11%)
Inguinal, leukemia mononuclear		1 (11%)			
Lumbar, leukemia mononuclear		3 (33%)	2 (20%)	1 (17%)	1 (11%)
Lumbar, sarcoma, metastatic, tissue NOS					1 (11%)
Mediastinal, leukemia mononuclear	2 (29%)	4 (44%)	3 (30%)	1 (17%)	2 (22%)
Pancreatic, leukemia mononuclear	3 (43%)	4 (44%)	2 (20%)	3 (50%)	
Renal, leukemia mononuclear	3 (43%)	3 (33%)	3 (30%)	2 (33%)	2 (22%)
Lymph node, mandibular	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear	6 (13%)	5 (10%)	7 (15%)	7 (15%)	6 (13%)
Lymphoma malignant				1 (2%)	1 (2%)
Lymph node, mesenteric	(48)	(47)	(48)	(47)	(48)
Leukemia mononuclear	5 (10%)	7 (15%)	8 (17%)	6 (13%)	6 (13%)
Lymphoma malignant				1 (2%)	1 (2%)
Spleen	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear	10 (21%)	19 (40%)	18 (38%)	15 (31%)	17 (35%)
Lymphoma malignant					1 (2%)
Thymus	(47)	(47)	(45)	(45)	(46)
Leukemia mononuclear	1 (2%)	3 (6%)	3 (7%)	3 (7%)	1 (2%)
Integumentary System					
Mammary gland	(48)	(48)	(46)	(47)	(48)
Adenocarcinoma	3 (6%)	1 (2%)	1 (2%)	4 (9%)	3 (6%)
Fibroadenoma	16 (33%)	18 (38%)	24 (52%)	22 (47%)	31 (65%)
Leukemia mononuclear			1 (2%)		
Skin	(48)	(48)	(48)	(48)	(48)
Basal cell carcinoma	2 (4%)				1 (2%)
Keratoacanthoma			1 (2%)		
Leukemia mononuclear					1 (2%)
Squamous cell papilloma		1 (2%)			
Ear, neural crest tumor, malignant				1 (2%)	
Subcutaneous tissue, fibroma	1 (2%)			1 (2%)	3 (6%)
Subcutaneous tissue, fibrosarcoma					1 (2%)
Subcutaneous tissue, sarcoma					1 (2%)
Subcutaneous tissue, sarcoma, metastatic, tissue NOS					1 (2%)
Tail, squamous cell papilloma					1 (2%)
Musculoskeletal System					
Bone	(0)	(0)	(0)	(0)	(1)
Cranium, osteoma					1 (100%)
Bone, femur	(48)	(48)	(48)	(48)	(48)
Skeletal muscle	(48)	(48)	(48)	(48)	(48)
Rhabdomyosarcoma				1 (2%)	
Sarcoma, metastatic, tissue NOS					1 (2%)
Subcutaneous tissue, schwannoma malignant		1 (2%)			
Nervous System					
Brain, brain stem	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear	1 (2%)		1 (2%)		
Brain, cerebellum	(48)	(48)	(48)	(48)	(48)
Astrocytoma NOS				1 (2%)	
Leukemia mononuclear	1 (2%)		2 (4%)	1 (2%)	

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Nervous System (continued)					
Brain, cerebrum	(48)	(48)	(48)	(48)	(48)
Astrocytoma NOS		1 (2%)		2 (4%)	
Leukemia mononuclear	1 (2%)		2 (4%)	1 (2%)	
Meninges, granular cell tumor NOS					1 (2%)
Peripheral nerve, sciatic	(48)	(48)	(48)	(48)	(48)
Spinal cord	(0)	(0)	(0)	(1)	(0)
Spinal cord, cervical	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear	1 (2%)		1 (2%)	1 (2%)	
Spinal cord, lumbar	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear	1 (2%)			2 (4%)	
Spinal cord, thoracic	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear	1 (2%)			1 (2%)	
Respiratory System					
Lung	(48)	(48)	(48)	(48)	(48)
Alveolar/bronchiolar adenoma	2 (4%)		1 (2%)		
Alveolar/bronchiolar adenoma, multiple	1 (2%)				
Carcinoma, metastatic, kidney			1 (2%)		
Histiocytic sarcoma					1 (2%)
Leukemia mononuclear	7 (15%)	9 (19%)	9 (19%)	11 (23%)	9 (19%)
Sarcoma stromal, metastatic, uterus		1 (2%)			
Nose	(47)	(48)	(48)	(48)	(48)
Leukemia mononuclear	1 (2%)		1 (2%)		
Special Senses System					
Eye	(45)	(48)	(47)	(45)	(46)
Leukemia mononuclear			1 (2%)		
Lids, melanoma malignant				1 (2%)	
Harderian gland	(48)	(48)	(48)	(48)	(48)
Histiocytic sarcoma					1 (2%)
Lacrimal gland	(0)	(1)	(1)	(0)	(1)
Zymbal's gland	(0)	(1)	(0)	(0)	(3)
Carcinoma		1 (100%)			
Squamous cell carcinoma					2 (67%)
Urinary System					
Kidney	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear	1 (2%)		1 (2%)		1 (2%)
Lymphoma malignant		1 (2%)			
Renal tubule, adenoma	1 (2%)				
Renal tubule, carcinoma			1 (2%)		
Transitional epithelium, carcinoma					1 (2%)
Urinary bladder	(48)	(48)	(48)	(48)	(47)
Leukemia mononuclear	1 (2%)	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Transitional epithelium, carcinoma					1 (2%)
Systemic Lesions					
Multiple organs	(48)[b]	(48)[b]	(48)[b]	(48)[b]	(48)[b]
Histiocytic sarcoma		1 (2%)			2 (4%)
Leukemia mononuclear	10 (21%)	19 (40%)	19 (40%)	15 (31%)	17 (35%)
Lymphoma malignant		1 (2%)		1 (2%)	1 (2%)
Mesothelioma malignant	1 (2%)				

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Neoplasm Summary					
Total animals with primary neoplasms[c]	46	48	48	45	46
Total primary neoplasms	103	123	123	115	151
Total animals with benign neoplasms	42	43	42	37	38
Total benign neoplasms	81	83	83	78	96
Total animals with malignant neoplasms	20	28	32	27	36
Total malignant neoplasms	22	39	40	34	54
Total animals with metastatic neoplasms		2	1		2
Total metastatic neoplasms		2	1		4
Total animals with neoplasms uncertain-benign or malignant		1		3	1
Total uncertain neoplasms		1		3	1

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

TABLE B2
Statistical Analysis of Neoplasms in Female Rats
in the 2-Year Drinking Water Study of Acrylamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Adrenal Medulla: Malignant Pheochromocytoma					
Overall rate[a]	2/48 (4%)	4/48 (8%)	1/48 (2%)	1/47 (2%)	2/48 (4%)
Adjusted rate[b]	4.6%	9.6%	2.6%	2.8%	6.2%
Terminal rate[c]	1/34 (3%)	2/28 (7%)	0/21 (0%)	1/23 (4%)	0/13 (0%)
First incidence (days)[d]	677	578	641	737 (T)	717
Poly-3 test[e]	P=0.505N	P=0.320	P=0.535N	P=0.567N	P=0.585
Adrenal Medulla: Malignant or Complex Pheochromocytoma					
Overall rate	2/48 (4%)	4/48 (8%)	1/48 (2%)	1/47 (2%)	3/48 (6%)
Adjusted rate	4.6%	9.6%	2.6%	2.8%	9.2%
Terminal rate	1/34 (3%)	2/28 (7%)	0/21 (0%)	1/23 (4%)	1/13 (8%)
First incidence (days)	677	578	641	737 (T)	717
Poly-3 test	P=0.426	P=0.320	P=0.535N	P=0.567N	P=0.372
Brain (Cerebellum or Cerebrum): Astrocytoma NOS					
Overall rate	0/48 (0%)	1/48 (2%)	0/48 (0%)	3/48 (6%)	0/48 (0%)
Adjusted rate	0%	2.4%	0%	8.2%	0%
Terminal rate	0/34 (0%)	1/28 (4%)	0/21 (0%)	2/23 (9%)	0/13 (0%)
First incidence (days)	-	737 (T)	-	564	-
Poly-3 test	P=0.408	P=0.491	-	P=0.092	-
Brain (Cerebral Meninges): Granular Cell Tumor NOS					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)
Adjusted rate	0%	0%	0%	0%	3.1%
Terminal rate	0/34 (0%)	0/28 (0%)	0/21 (0%)	0/23 (0%)	0/13 (0%)
First incidence (days)	-	-	-	-	730
Poly-3 test	P=0.121	-	-	-	P=0.444
Clitoral Gland: Adenoma					
Overall rate	9/48 (19%)	7/48 (15%)	5/47 (11%)	8/48 (17%)	3/47 (6%)
Adjusted rate	20.6%	16.8%	12.6%	21.4%	9.4%
Terminal rate	7/34 (21%)	5/28 (18%)	1/21 (5%)	4/23 (17%)	2/13 (15%)
First incidence (days)	656	655	402	564	564
Poly-3 test	P=0.214N	P=0.431N	P=0.247N	P=0.573	P=0.162N
Clitoral Gland: Carcinoma					
Overall rate	1/48 (2%)	6/48 (13%)	12/47 (26%)	3/48 (6%)	8/47 (17%)
Adjusted rate	2.3%	14.4%	30.3%	8.1%	24.4%
Terminal rate	1/34 (3%)	2/28 (7%)	5/21 (24%)	1/23 (4%)	2/13 (15%)
First incidence (days)	737 (T)	676	632	585	416
Poly-3 test	P=0.046	P=0.050	P<0.001	P=0.253	P=0.004
Clitoral Gland: Adenoma or Carcinoma					
Overall rate	10/48 (21%)	13/48 (27%)	17/47 (36%)	11/48 (23%)	11/47 (23%)
Adjusted rate	22.9%	30.9%	41.3%	28.8%	33.0%
Terminal rate	8/34 (24%)	7/28 (25%)	6/21 (29%)	5/23 (22%)	4/13 (31%)
First incidence (days)	656	655	402	564	416
Poly-3 test	P=0.323	P=0.277	P=0.053	P=0.363	P=0.236
Clitoral Gland: Squamous Cell Carcinoma					
Overall rate	0/48 (0%)	0/48 (0%)	0/47 (0%)	1/48 (2%)	0/47 (0%)
Adjusted rate	0%	0%	0%	2.8%	0%
Terminal rate	0/34 (0%)	0/28 (0%)	0/21 (0%)	1/23 (4%)	0/13 (0%)
First incidence (days)	-	-	-	737 (T)	-
Poly-3 test	P=0.517	-	-	P=0.465	-

TABLE B2
Statistical Analysis of Neoplasms in Female Rats
in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Clitoral Gland: Squamous Cell Papilloma					
Overall rate	0/48 (0%)	0/48 (0%)	1/47 (2%)	0/48 (0%)	3/47 (6%)
Adjusted rate	0%	0%	2.6%	0%	9.3%
Terminal rate	0/34 (0%)	0/28 (0%)	0/21 (0%)	0/23 (0%)	1/13 (8%)
First incidence (days)	-	-	726	-	418
Poly-3 test	P=0.010	-	P=0.475	-	P=0.075
Clitoral Gland: Squamous Cell Carcinoma or Papilloma, Adenoma or Carcinoma					
Overall rate	10/48 (21%)	13/48 (27%)	17/47 (36%)	12/48 (25%)	14/47 (30%)
Adjusted rate	22.9%	30.9%	41.3%	31.4%	40.8%
Terminal rate	8/34 (24%)	7/28 (25%)	6/21 (29%)	6/23 (26%)	5/13 (39%)
First incidence (days)	656	655	402	564	416
Poly-3 test	P=0.102	P=0.277	P=0.053	P=0.270	P=0.071
Heart: Malignant Schwannoma					
Overall rate	2/48 (4%)	1/48 (2%)	0/48 (0%)	2/48 (4%)	4/48 (8%)
Adjusted rate	4.6%	2.4%	0%	5.5%	12.3%
Terminal rate	2/34 (6%)	1/28 (4%)	0/21 (0%)	1/23 (4%)	2/13 (15%)
First incidence (days)	737 (T)	737 (T)	-	613	723
Poly-3 test	P=0.047	P=0.515N	P=0.261N	P=0.634	P=0.217
Liver: Hepatocellular Adenoma					
Overall rate	0/48 (0%)	0/48 (0%)	1/48 (2%)	1/48 (2%)	3/48 (6%)
Adjusted rate	0%	0%	2.6%	2.8%	9.3%
Terminal rate	0/34 (0%)	0/28 (0%)	0/21 (0%)	1/23 (4%)	2/13 (15%)
First incidence (days)	-	-	725	737 (T)	709
Poly-3 test	P=0.010	-	P=0.479	P=0.465	P=0.076
Lung: Alveolar/bronchiolar Adenoma					
Overall rate	3/48 (6%)	0/48 (0%)	1/48 (2%)	0/48 (0%)	0/48 (0%)
Adjusted rate	7.0%	0%	2.6%	0%	0%
Terminal rate	3/34 (9%)	0/28 (0%)	1/21 (5%)	0/23 (0%)	0/13 (0%)
First incidence (days)	737 (T)	-	737 (T)	-	-
Poly-3 test	P=0.081N	P=0.126N	P=0.343N	P=0.153N	P=0.179N
Mammary Gland: Fibroadenoma					
Overall rate	16/48 (33%)	18/48 (38%)	24/46 (52%)	22/47 (47%)	31/48 (65%)
Adjusted rate	36.4%	42.2%	59.0%	58.7%	84.2%
Terminal rate	12/34 (35%)	13/28 (46%)	12/21 (57%)	16/23 (70%)	13/13 (100%)
First incidence (days)	656	579	376	501	474
Poly-3 test	P<0.001	P=0.371	P=0.027	P=0.033	P<0.001
Mammary Gland: Adenocarcinoma					
Overall rate	3/48 (6%)	1/48 (2%)	1/46 (2%)	4/47 (9%)	3/48 (6%)
Adjusted rate	6.9%	2.4%	2.7%	11.1%	9.3%
Terminal rate	2/34 (6%)	1/28 (4%)	0/21 (0%)	2/23 (9%)	3/13 (23%)
First incidence (days)	670	737 (T)	641	694	737 (T)
Poly-3 test	P=0.186	P=0.323N	P=0.358N	P=0.398	P=0.521
Mammary Gland: Fibroadenoma or Adenocarcinoma					
Overall rate	17/48 (35%)	18/48 (38%)	25/46 (54%)	22/47 (47%)	31/48 (65%)
Adjusted rate	38.5%	42.2%	60.9%	58.7%	84.2%
Terminal rate	12/34 (35%)	13/28 (46%)	12/21 (57%)	16/23 (70%)	13/13 (100%)
First incidence (days)	656	579	376	501	474
Poly-3 test	P<0.001	P=0.448	P=0.027	P=0.050	P<0.001

TABLE B2
Statistical Analysis of Neoplasms in Female Rats
in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Oral Mucosa: Squamous Cell Carcinoma[f]					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)
Adjusted rate	0%	0%	0%	0%	3.0%
Terminal rate	0/34 (0%)	0/28 (0%)	0/21 (0%)	0/23 (0%)	0/13 (0%)
First incidence (days)	-	-	-	-	474
Poly-3 test	P=0.123	-	-	-	P=0.448
Oral Mucosa: Squamous Cell Papilloma[f]					
Overall rate	0/48 (0%)	2/48 (4%)	1/48 (2%)	2/48 (4%)	4/48 (8%)
Adjusted rate	0%	4.8%	2.6%	5.5%	12.3%
Terminal rate	0/34 (0%)	1/28 (4%)	1/21 (5%)	1/23 (4%)	2/13 (15%)
First incidence (days)	-	519	737 (T)	663	681
Poly-3 test	P=0.016	P=0.231	P=0.479	P=0.202	P=0.032
Tongue: Squamous Cell Carcinoma[f]					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)
Adjusted rate	0%	0%	0%	0%	3.0%
Terminal rate	0/34 (0%)	0/28 (0%)	0/21 (0%)	0/23 (0%)	0/13 (0%)
First incidence (days)	-	-	-	-	474
Poly-3 test	P=0.123	-	-	-	P=0.448
Tongue: Squamous Cell Papilloma[f]					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)	1/48 (2%)
Adjusted rate	0%	0%	0%	2.7%	3.1%
Terminal rate	0/34 (0%)	0/28 (0%)	0/21 (0%)	0/23 (0%)	1/13 (8%)
First incidence (days)	-	-	-	682	737 (T)
Poly-3 test	P=0.111	-	-	P=0.466	P=0.443
Oral Mucosa or Tongue: Squamous Cell Papilloma[f]					
Overall rate	0/48 (0%)	2/48 (4%)	1/48 (2%)	3/48 (6%)	4/48 (8%)
Adjusted rate	0%	4.8%	2.6%	8.2%	12.3%
Terminal rate	0/34 (0%)	1/28 (4%)	1/21 (5%)	1/23 (4%)	2/13 (15%)
First incidence (days)	-	519	737 (T)	663	681
Poly-3 test	P=0.014	P=0.231	P=0.479	P=0.092	P=0.032
Oral Mucosa or Tongue: Squamous Cell Papilloma or Squamous Cell Carcinoma[f]					
Overall rate	0/48 (0%)	2/48 (4%)	1/48 (2%)	3/48 (6%)	5/48 (10%)
Adjusted rate	0%	4.8%	2.6%	8.2%	15.0%
Terminal rate	0/34 (0%)	1/28 (4%)	1/21 (5%)	1/23 (4%)	2/13 (15%)
First incidence (days)	-	519	737 (T)	663	474
Poly-3 test	P=0.004	P=0.231	P=0.479	P=0.092	P=0.014
Pituitary Gland (Pars Distalis): Adenoma					
Overall rate	37/48 (77%)	35/48 (73%)	33/47 (70%)	29/48 (60%)	28/48 (58%)
Adjusted rate	81.0%	78.1%	78.8%	74.0%	75.0%
Terminal rate	28/34 (82%)	24/28 (86%)	16/20 (80%)	19/23 (83%)	11/13 (85%)
First incidence (days)	546	513	563	501	418
Poly-3 test	P=0.252N	P=0.465N	P=0.504N	P=0.290N	P=0.334N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma					
Overall rate	37/48 (77%)	35/48 (73%)	33/47 (70%)	30/48 (63%)	28/48 (58%)
Adjusted rate	81.0%	78.1%	78.8%	76.4%	75.0%
Terminal rate	28/34 (82%)	24/28 (86%)	16/20 (80%)	19/23 (83%)	11/13 (85%)
First incidence (days)	546	513	563	501	418
Poly-3 test	P=0.279N	P=0.465N	P=0.504N	P=0.395N	P=0.334N

TABLE B2
Statistical Analysis of Neoplasms in Female Rats
in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Skin (Subcutaneous Tissue): Fibrosarcoma or Sarcoma					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	2/48 (4%)
Adjusted rate	0%	0%	0%	0%	6.2%
Terminal rate	0/34 (0%)	0/28 (0%)	0/21 (0%)	0/23 (0%)	1/13 (8%)
First incidence (days)	-	-	-	-	719
Poly-3 test	P=0.017	-	-	-	P=0.180
Skin (Subcutaneous Tissue): Fibroma	1/48 (2%)	0/48 (0%)	0/48 (0%)	1/48 (2%)	3/48 (6%)
Overall rate	2.3%	0%	0%	2.8%	9.3%
Adjusted rate	1/34 (3%)	0/28 (0%)	0/21 (0%)	1/23 (4%)	2/13 (15%)
Terminal rate	737 (T)	-	-	737 (T)	724
First incidence (days)	P=0.027	P=0.509N	P=0.521N	P=0.720	P=0.212
Poly-3 test					
Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, or Sarcoma					
Overall rate	1/48 (2%)	0/48 (0%)	0/48 (0%)	1/48 (2%)	5/48 (10%)
Adjusted rate	2.3%	0%	0%	2.8%	15.4%
Terminal rate	1/34 (3%)	0/28 (0%)	0/21 (0%)	1/23 (4%)	3/13 (23%)
First incidence (days)	737 (T)	-	-	737 (T)	719
Poly-3 test	P=0.001	P=0.509N	P=0.521N	P=0.720	P=0.050
Skin: All Morphologies					
Overall rate	3/48 (6%)	1/48 (2%)	1/48 (2%)	2/48 (4%)	7/48 (15%)
Adjusted rate	6.9%	2.4%	2.6%	5.5%	21.2%
Terminal rate	2/34 (6%)	1/28 (4%)	1/21 (5%)	2/23 (9%)	4/13 (31%)
First incidence (days)	565	737 (T)	737 (T)	737 (T)	548
Poly-3 test	P=0.006	P=0.326N	P=0.348N	P=0.585N	P=0.067
Stomach (Forestomach): Squamous Cell Papilloma					
Overall rate	0/48 (0%)	0/48 (0%)	2/48 (4%)	0/48 (0%)	0/48 (0%)
Adjusted rate	0%	0%	5.1%	0%	0%
Terminal rate	0/34 (0%)	0/28 (0%)	0/21 (0%)	0/23 (0%)	0/13 (0%)
First incidence (days)	-	-	651	-	-
Poly-3 test	P=0.571N	-	P=0.216	-	-
Thyroid Gland: C-Cell Adenoma					
Overall rate	1/48 (2%)	4/48 (8%)	4/48 (8%)	3/48 (6%)	4/47 (9%)
Adjusted rate	2.3%	9.7%	10.3%	8.2%	12.3%
Terminal rate	0/34 (0%)	3/28 (11%)	4/21 (19%)	2/23 (9%)	1/13 (8%)
First incidence (days)	689	693	737 (T)	611	605
Poly-3 test	P=0.152	0.165	P=0.147	P=0.248	P=0.105
Thyroid Gland: C-Cell Carcinoma					
Overall rate	1/48 (2%)	0/48 (0%)	2/48 (4%)	0/48 (0%)	0/47 (0%)
Adjusted rate	2.3%	0%	5.1%	0%	0.0%
Terminal rate	1/34 (3%)	0/28 (0%)	2/21 (10%)	0/23 (0%)	0/13 (0%)
First incidence (days)	737 (T)	-	737 (T)	-	-
Poly-3 test	P=0.322N	P=0.509N	P=0.464	P=0.535N	P=0.559N
Thyroid Gland: C-Cell Adenoma or Carcinoma					
Overall rate	2/48 (4%)	4/48 (8%)	6/48 (13%)	3/48 (6%)	4/47 (9%)
Adjusted rate	4.6%	9.7%	15.4%	8.2%	12.3%
Terminal rate	1/34 (3%)	3/28 (11%)	6/21 (29%)	2/23 (9%)	1/13 (8%)
First incidence (days)	689	693	737 (T)	611	605
Poly-3 test	P=0.273	P=0.316	P=0.099	P=0.424	P=0.218

TABLE B2
Statistical Analysis of Neoplasms in Female Rats
in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Thyroid Gland: Follicular Cell Adenoma					
Overall rate	0/48 (0%)	0/48 (0%)	1/48 (2%)	0/48 (0%)	2/47 (4%)
Adjusted rate	0%	0%	2.6%	0%	6.3%
Terminal rate	0/34 (0%)	0/28 (0%)	1/21 (5%)	0/23 (0%)	1/13 (8%)
First incidence (days)	-	-	737 (T)	-	724
Poly-3 test	P=0.052	-	P=0.479	-	P=0.177
Thyroid Gland: Follicular Cell Carcinoma					
Overall rate	0/48 (0%)	0/48 (0%)	1/48 (2%)	3/48 (6%)	2/47 (4%)
Adjusted rate	0%	0%	2.6%	8.2%	6.3%
Terminal rate	0/34 (0%)	0/28 (0%)	0/21 (0%)	2/23 (9%)	2/13 (15%)
First incidence (days)	-	-	642	679	737 (T)
Poly-3 test	P=0.031	-	P=0.481	P=0.091	P=0.177
Thyroid Gland: Follicular Cell Adenoma or Carcinoma					
Overall rate	0/48 (0%)	0/48 (0%)	2/48 (4%)	3/48 (6%)	4/47 (9%)
Adjusted rate	0%	0%	5.1%	8.2%	12.5%
Terminal rate	0/34 (0%)	0/28 (0%)	1/21 (5%)	2/23 (9%)	3/13 (23%)
First incidence (days)	-	-	642	679	724
Poly-3 test	P=0.003	-	P=0.216	P=0.091	P=0.031
Thyroid Gland: C-Cell or Follicular Cell Adenoma or Carcinoma					
Overall rate	2/48 (4%)	4/48 (8%)	8/48 (17%)	6/48 (13%)	8/47 (17%)
Adjusted rate	4.6%	9.7%	20.4%	16.3%	24.5%
Terminal rate	1/34 (3%)	3/28 (11%)	7/21 (33%)	4/23 (17%)	4/13 (31%)
First incidence (days)	689	693	642	611	605
Poly-3 test	P=0.012	P=0.316	P=0.029	P=0.086	P=0.013
Uterus: Stromal Polyp					
Overall rate	9/48 (19%)	12/48 (25%)	9/48 (19%)	10/48 (21%)	12/48 (25%)
Adjusted rate	20.6%	28.4%	22.4%	26.2%	33.9%
Terminal rate	7/34 (21%)	8/28 (29%)	5/21 (24%)	5/23 (22%)	5/13 (39%)
First incidence (days)	565	599	586	428	544
Poly-3 test	P=0.143	P=0.275	P=0.524	P=0.370	P=0.140
Uterus: Stromal Sarcoma					
Overall rate	0/48 (0%)	4/48 (8%)	3/48 (6%)	0/48 (0%)	4/48 (8%)
Adjusted rate	0%	9.6%	7.5%	0%	11.9%
Terminal rate	0/34 (0%)	1/28 (4%)	0/21 (0%)	0/23 (0%)	0/13 (0%)
First incidence (days)	-	704	579	-	550
Poly-3 test	P=0.139	P=0.055	P=0.104	-	P=0.035
Uterus: Stromal Polyp or Sarcoma					
Overall rate	9/48 (19%)	16/48 (33%)	12/48 (25%)	10/48 (21%)	16/48 (33%)
Adjusted rate	20.6%	37.7%	29.2%	26.2%	43.5%
Terminal rate	7/34 (21%)	9/28 (32%)	5/21 (24%)	5/23 (22%)	5/13 (39%)
First incidence (days)	565	599	579	428	544
Poly-3 test	P=0.063	P=0.062	P=0.252	P=0.370	P=0.022
Zymbal's Gland: Squamous Cell Carcinoma[f]					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	2/48 (4%)
Adjusted rate	0%	0%	0%	0%	5.9%
Terminal rate	0/34 (0%)	0/28 (0%)	0/21 (0%)	0/23 (0%)	0/13 (0%)
First incidence (days)	-	-	-	-	285
Poly-3 test	P=0.018	-	-	-	P=0.187

TABLE B2
Statistical Analysis of Neoplasms in Female Rats
in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
All Organs: Histiocytic Sarcoma					
Overall rate	0/48 (0%)	1/48 (2%)	0/48 (0%)	0/48 (0%)	2/48 (4%)
Adjusted rate	0%	2.4%	0%	0%	6.1%
Terminal rate	0/34 (0%)	0/28 (0%)	0/21 (0%)	0/23 (0%)	1/13 (8%)
First incidence (days)	-	522	-	-	599
Poly-3 test	P=0.086	P=0.494	-	-	P=0.182
All Organs: Leukemia					
Overall rate	10/48 (21%)	19/48 (40%)	19/48 (40%)	15/48 (31%)	17/48 (35%)
Adjusted rate	21.9%	43.0%	44.1%	37.0%	46.5%
Terminal rate	4/34 (12%)	9/28 (32%)	4/21 (19%)	5/23 (22%)	7/13 (54%)
First incidence (days)	463	513	564	286	424
Poly-3 test	P=0.064	P=0.026	P=0.020	P=0.096	P=0.015
All Organs: Malignant Lymphoma					
Overall rate	0/48 (0%)	1/48 (2%)	0/48 (0%)	1/48 (2%)	1/48 (2%)
Adjusted rate	0%	2.4%	0%	2.7%	3.0%
Terminal rate	0/34 (0%)	1/28 (4%)	0/21 (0%)	0/23 (0%)	0/13 (0%)
First incidence (days)	-	737 (T)	-	464	480
Poly-3 test	P=0.265	P=0.491	-	P=0.469	P=0.448
All Organs: Mesothelioma					
Overall rate	1/48 (2%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/48 (0%)
Adjusted rate	2.3%	0%	0%	0%	0%
Terminal rate	0/34 (0%)	0/28 (0%)	0/21 (0%)	0/23 (0%)	0/13 (0%)
First incidence (days)	699	-	-	-	-
Poly-3 test	P=0.328N	P=0.509N	P=0.521N	P=0.535N	P=0.557N
All Organs: Osteosarcoma or Osteoma					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)
Adjusted rate	0%	0%	0%	0%	3.1%
Terminal rate	0/34 (0%)	0/28 (0%)	0/21 (0%)	0/23 (0%)	1/13 (8%)
First incidence (days)	-	-	-	-	737 (T)
Poly-3 test	P=0.121	-	-	-	P=0.443
All Organs: Benign Neoplasms					
Overall rate	42/48 (88%)	43/48 (90%)	42/48 (88%)	37/48 (77%)	38/48 (79%)
Adjusted rate	91.1%	92.9%	91.9%	89.0%	93.0%
Terminal rate	31/34 (91%)	28/28 (100%)	21/21 (100%)	22/23 (96%)	13/13 (100%)
First incidence (days)	546	513	376	428	418
Poly-3 test	P=0.538	P=0.536	P=0.609	P=0.513N	P=0.538
All Organs: Malignant Neoplasms					
Overall rate	20/48 (42%)	28/48 (58%)	32/48 (67%)	27/48 (56%)	36/48 (75%)
Adjusted rate	42.8%	61.7%	72.1%	62.3%	83.7%
Terminal rate	10/34 (29%)	13/28 (46%)	11/21 (52%)	10/23 (44%)	12/13 (92%)
First incidence (days)	463	513	564	286	285
Poly-3 test	P<0.001	P=0.051	P=0.003	P=0.047	P<0.001
All Organs: Benign and Malignant Neoplasms					
Overall rate	46/48 (96%)	48/48 (100%)	48/48 (100%)	45/48 (94%)	46/48 (96%)
Adjusted rate	95.8%	100.0%	100.0%	97.1%	99.5%
Terminal rate	32/34 (94%)	28/28 (100%)	21/21 (100%)	22/23 (96%)	13/13 (100%)
First incidence (days)	463	513	376	286	285
Poly-3 test	P=0.394	P=0.237	P=0.237	P=0.587	P=0.307

TABLE B2
Statistical Analysis of Neoplasms in Female Rats
in the 2-Year Drinking Water Study of Acrylamide (continued)

[a] Number of animals with neoplasm per number of animals examined microscopically.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[c] Observed incidence at the terminal sacrifice.
[d] T indicates terminal sacrifice.
[e] Beneath the 0 mM acrylamide are the p values associated with the trend test. Beneath the treated (0.0875, 0.175, 0.35, and 0.70 mM acrylamide) group incidences are the p values corresponding to pairwise comparisons between the 0 mM acrylamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice. An N indicates a negative trend or decreased tumor incidence.
[f] Results based on gross pathology.

TABLE B3a
Historical Incidence of Thyroid Gland Follicular Cell Neoplasms in NCTR Control Female F344/N Rats

		Incidence in Controls	
Study (Report Date)	**Route of Administration**	**Carcinoma**	**Adenoma or Carcinoma**
Doxylamine (April 1991)	Diet	0/47 (0.0%)	0/47 (0.0%)
Fumonisin B_1 (March 1999)	Diet	0/48 (0.0%)	0/48 (0.0%)
Gentian Violet (November 1988)	Diet	0/159 (0.0%)	1/159 (0.6%)
Leucomalachite Green (June 2001)	Diet	0/46 (0.0%)	0/46 (0.0%)
Malachite Green (June 2001)	Diet	0/46 (0.0%)	0/46 (0.0%)
Pyrilamine (July 1991)	Diet	0/48 (0.0%)	0/48 (0.0%)
Sulfamethazine (February 1988)	Diet	0/170 (0.0%)	5/170 (2.9%)
Triprolidine (June 1991)	Diet	0/45 (0.0%)	1/45 (2.2%)
Total (%)		0/609 (0.0%)	7/609 (1.1%)
Range		0%	0.0%-2.9%

TABLE B3b
Historical Incidence of Carcinoma of the Clitoral Gland in NCTR Control Female F344/N Rats

Study (Report Date)	**Route of Administration**	**Incidence in Controls**
Doxylamine (April 1991)	Diet	3/46 (6.5%)
Fumonisin B_1 (March 1999)	Diet	1/41 (2.4%)
Leucomalachite Green (June 2001)	Diet	2/47 (4.3%)
Malachite Green (June 2001)	Diet	5/48 (10.4%)
Pyrilamine (July 1991)	Diet	3/45 (6.7%)
Triprolidine (June 1991)	Diet	0/46 (0.0%)
Total (%)		14/273 (5.1%)
Range		0.0%-10.4%

TABLE B3c
Historical Incidence of Fibroadenoma of the Mammary Gland in NCTR Control Female F344/N Rats

Study (Report Date)	Route of Administration	Incidence in Controls
Doxylamine (April 1991)	Diet	19/48 (39.6%)
Fumonisin B_1 (March 1999)	Diet	18/47 (38.3%)
Gentian Violet (November 1988)	Diet	65/169 (38.5%)
Leucomalachite Green (June 2001)	Diet	20/48 (41.7%)
Malachite Green (June 2001)	Diet	15/46 (32.6%)
Pyrilamine (July 1991)	Diet	20/47 (42.6%)
Sulfamethazine (February 1988)	Diet	48/177 (27.1%)
Triprolidine (June 1991)	Diet	15/46 (32.6%)
Total (%)		220/628 (35.0%)
Range		27.1%-42.6%

TABLE B3d
Historical Incidence of Squamous Cell Carcinoma or Papilloma (Combined) of the Oral Cavity in NCTR Control Female F344/N Rats

Study (Report Date)	Route of Administration	Incidence in Controls
Doxylamine (April 1991)	Diet	-[a]
Fumonisin B_1 (March 1999)	Diet	0/48 (0.0%)
Gentian Violet (November 1988)	Diet	1/167 (0.6%)
Leucomalachite Green (June 2001)	Diet	0/48 (0.0%)
Malachite Green (June 2001)	Diet	0/48 (0.0%)
Pyrilamine (July 1991)	Diet	1/48 (2.1%)
Sulfamethazine (February 1988)	Diet	0/179 (0.0%)
Triprolidine (June 1991)	Diet	-
Total (%)		2/538 (0.4%)
Range		0.0%-2.1%

[a] Not reported.

TABLE B3e
Historical Incidence of Skin Fibroma, Fibrosarcoma, Myxoma, Myxosarcoma, or Fibrous Histiocytoma in NCTR Control Female F344/N Rats

Study (Report Date)	Route of Administration	Incidence in Controls
Doxylamine (April 1991)	Diet	1/48 (2.1%)
Fumonisin B_1 (March 1999)	Diet	1/48 (2.1%)
Gentian Violet (November 1988)	Diet	2/165 (1.2%)
Leucomalachite Green (June 2001)	Diet	0/48 (0.0%)
Malachite Green (June 2001)	Diet	0/48 (0.0%)
Pyrilamine (July 1991)	Diet	0/47 (0.0%)
Sulfamethazine (February 1988)	Diet	2/179 (1.1%)
Triprolidine (June 1991)	Diet	0/47 (0.0%)
Total (%)		6/630 (1.0%)
Range		0.0%-2.1%

TABLE B3f
Historical Incidence of Malignant Schwannoma of the Heart in NCTR Control Female F344/N Rats

Study (Report Date)	Route of Administration	Incidence in Controls
Doxylamine (April 1991)	Diet	0/48 (0.0%)
Fumonisin B_1 (March 1999)	Diet	0/48 (0.0%)
Gentian Violet (November 1988)	Diet	0/169 (0.0%)
Leucomalachite Green (June 2001)	Diet	0/48 (0.0%)
Malachite Green (June 2001)	Diet	0/48 (0.0%)
Pyrilamine (July 1991)	Diet	0/48 (0.0%)
Sulfamethazine (February 1988)	Diet	0/179 (0.0%)
Triprolidine (June 1991)	Diet	0/48 (0.0%)
Total (%)		0/636 (0.0%)
Range		0.0%

TABLE B3g
Historical Incidence of Liver Hepatocellular Adenoma in NCTR Control Female F344/N Rats

Study (Report Date)	Route of Administration	Incidence in Controls
Doxylamine (April 1991)	Diet	0/48 (0.0%)
Fumonisin B_1 (March 1999)	Diet	0/48 (0.0%)
Gentian Violet (November 1988)	Diet	0/170 (0.0%)
Leucomalachite Green (June 2001)	Diet	1/48 (2.1%)
Malachite Green (June 2001)	Diet	0/48 (0.0%)
Pyrilamine (July 1991)	Diet	0/48 (0.0%)
Sulfamethazine (February 1988)	Diet	1/179 (0.6%)
Triprolidine (June 1991)	Diet	0/48 (0.0%)
Total (%)		2/637 (0.3%)
Range		0.0%-2.1%

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Acrylamide[a]

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Disposition Summary					
Animals initially in study	48	48	48	48	48
Early deaths					
Moribund sacrifice	8	13	17	14	23
Natural deaths	3	2	2	5	2
Survivors					
Moribund sacrifice	2	5	7	6	10
Natural deaths	1		1		
Terminal sacrifice	34	28	21	23	13
Animals examined microscopically	48	48	48	48	48
Alimentary System					
Esophagus	(48)	(48)	(48)	(48)	(48)
Inflammation					1 (2%)
Intestine large, cecum	(47)	(48)	(48)	(46)	(47)
Lymphoid tissue, hyperplasia	1 (2%)	1 (2%)			
Mucosa, hyperplasia	1 (2%)				
Intestine large, colon	(46)	(48)	(48)	(47)	(47)
Diverticulum		1 (2%)			
Intestine small, duodenum	(48)	(48)	(48)	(46)	(47)
Intestine small, ileum	(47)	(48)	(48)	(46)	(46)
Lymphoid tissue, hyperplasia	2 (4%)	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Intestine small, jejunum	(46)	(48)	(48)	(45)	(46)
Keratin cyst					1 (2%)
Liver	(48)	(48)	(48)	(48)	(48)
Angiectasis	1 (2%)	2 (4%)	3 (6%)	1 (2%)	
Apoptosis					1 (2%)
Basophilic focus	1 (2%)		1 (2%)	2 (4%)	
Basophilic focus, multiple	29 (60%)	25 (52%)	25 (52%)	23 (48%)	19 (40%)
Degeneration, cystic			1 (2%)	2 (4%)	2 (4%)
Eosinophilic focus	7 (15%)	3 (6%)	8 (17%)	7 (15%)	1 (2%)
Eosinophilic focus, multiple	1 (2%)	2 (4%)	1 (2%)	4 (8%)	3 (6%)
Granuloma	18 (38%)	15 (31%)	15 (31%)	18 (38%)	11 (23%)
Hematopoietic cell proliferation	1 (2%)		4 (8%)	2 (4%)	5 (10%)
Hepatodiaphragmatic nodule	3 (6%)	4 (8%)	2 (4%)	1 (2%)	3 (6%)
Infiltration cellular, lymphocyte	1 (2%)	1 (2%)			3 (6%)
Inflammation		1 (2%)			1 (2%)
Necrosis, coagulative			2 (4%)		
Regeneration	1 (2%)	2 (4%)			2 (4%)
Tension lipidosis					1 (2%)
Thrombosis			1 (2%)		
Vacuolization cytoplasmic	7 (15%)	6 (13%)	7 (15%)	3 (6%)	4 (8%)
Bile duct, hyperplasia	7 (15%)	8 (17%)	4 (8%)	4 (8%)	5 (10%)
Biliary tract, cyst	1 (2%)				
Capsule, fibrosis		1 (2%)	1 (2%)		
Centrilobular, cytoplasmic alteration				1 (2%)	1 (2%)
Centrilobular, necrosis	2 (4%)	1 (2%)	1 (2%)	1 (2%)	3 (6%)
Hepatocyte, hyperplasia			1 (2%)		3 (6%)
Left lateral lobe, developmental malformation	9 (19%)	10 (21%)	4 (8%)	6 (13%)	2 (4%)
Median lobe, developmental malformation	1 (2%)	1 (2%)		1 (2%)	
Oval cell, hyperplasia					1 (2%)
Right lateral lobe, developmental malformation	1 (2%)	1 (2%)			1 (2%)
Mesentery	(7)	(9)	(10)	(4)	(5)
Accessory spleen			1 (10%)		
Fat, necrosis	5 (71%)	9 (100%)	6 (60%)	4 (100%)	4 (80%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Alimentary System (continued)					
Oral Mucosa	(0)	(2)	(2)	(3)	(7)
Hyperplasia				1 (33%)	2 (29%)
Pancreas	(48)	(48)	(48)	(47)	(48)
Accessory Spleen	1 (2%)			1 (2%)	
Infiltration cellular, lymphocyte	1 (2%)				
Inflammation					2 (4%)
Polyarteritis		1 (2%)			
Acinar cell, atrophy	20 (42%)	16 (33%)	10 (21%)	6 (13%)	8 (17%)
Salivary glands	(48)	(48)	(48)	(48)	(48)
Stomach, forestomach	(48)	(48)	(48)	(48)	(48)
Edema	1 (2%)	2 (4%)		1 (2%)	1 (2%)
Hyperplasia	1 (2%)	3 (6%)	1 (2%)	3 (6%)	2 (4%)
Inflammation	1 (2%)		2 (4%)		1 (2%)
Ulcer		1 (2%)	1 (2%)	1 (2%)	2 (4%)
Stomach, glandular	(48)	(48)	(48)	(48)	(48)
Edema			1 (2%)		
Hemorrhage					1 (2%)
Inflammation			1 (2%)		1 (2%)
Tongue	(0)	(0)	(0)	(1)	(3)
Hyperplasia					1 (33%)
Cardiovascular System					
Heart	(48)	(48)	(48)	(48)	(48)
Cardiomyopathy	35 (73%)	42 (88%)	26 (54%)	37 (77%)	30 (63%)
Infiltration cellular, lymphocyte	1 (2%)				
Inflammation					1 (2%)
Mineralization	1 (2%)				
Atrium, thrombosis	3 (6%)			1 (2%)	3 (6%)
Myocardium, hyperplasia					1 (2%)
Endocrine System					
Adrenal cortex	(48)	(48)	(48)	(48)	(48)
Accessory adrenal cortical nodule	1 (2%)				1 (2%)
Angiectasis	37 (77%)	29 (54%)	29 (60%)	23 (48%)	20 (42%)
Atrophy	1 (2%)	1 (2%)			
Hematopoietic cell proliferation	1 (2%)				
Hyperplasia, focal		1 (2%)	2 (4%)	1 (2%)	2 (4%)
Hypertrophy, focal	4 (8%)	5 (10%)	5 (10%)	4 (8%)	10 (21%)
Infarct		1 (2%)			
Thrombosis					1 (2%)
Vacuolization cytoplasmic	2 (4%)	5 (10%)	5 (10%)	5 (10%)	9 (19%)
Adrenal medulla	(48)	(48)	(48)	(47)	(48)
Angiectasis	1 (2%)		1 (2%)	1 (2%)	
Hyperplasia, focal	3 (6%)		1 (2%)	1 (2%)	1 (2%)
Hypertrophy, focal		1 (2%)			
Islets, pancreatic	(48)	(48)	(48)	(47)	(48)
Parathyroid gland	(47)	(47)	(45)	(46)	(46)
Pituitary gland	(48)	(48)	(47)	(48)	(48)
Angiectasis	1 (2%)		2 (4%)	2 (4%)	
Pars distalis, cyst	2 (4%)	2 (4%)	2 (4%)	1 (2%)	4 (8%)
Pars distalis, hyperplasia	4 (8%)	8 (17%)	1 (2%)	4 (8%)	4 (8%)
Pars intermedia, cyst		1 (2%)	1 (2%)		
Pars nervosa, cyst	1 (2%)				
Thyroid gland	(48)	(48)	(48)	(48)	(47)
C-cell, hyperplasia	15 (31%)	4 (8%)	5 (10%)	6 (13%)	6 (13%)
Follicle, cyst		1 (2%)		1 (2%)	1 (2%)
Follicular cell, hyperplasia				1 (2%)	2 (4%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
General Body System					
Tissue NOS	(0)	(0)	(1)	(0)	(1)
Genital System					
Clitoral gland	(48)	(48)	(47)	(48)	(47)
Hyperplasia				1 (2%)	1 (2%)
Inflammation	29 (60%)	24 (50%)	22 (47%)	28 (58%)	22 (47%)
Duct, ectasia	12 (25%)	10 (21%)	9 (19%)	10 (21%)	16 (34%)
Ovary	(48)	(48)	(48)	(48)	(48)
Atrophy	38 (79%)	41 (85%)	43 (90%)	44 (92%)	43 (90%)
Cyst	4 (8%)	5 (10%)	3 (6%)	3 (6%)	5 (10%)
Infiltration cellular, histiocyte	1 (2%)				
Infiltration cellular, lymphocyte	1 (2%)				
Uterus	(48)	(48)	(48)	(48)	(48)
Angiectasis			1 (2%)		
Inflammation	1 (2%)	1 (2%)		2 (4%)	
Prolapse				1 (2%)	
Thrombosis	1 (2%)				
Bilateral, horn, dilatation		1 (2%)			
Cervix, mucocyte, metaplasia			1 (2%)		1 (2%)
Cervix, muscularis, hypertrophy		1 (2%)			
Endometrial glands, hyperplasia		2 (4%)	1 (2%)		
Endometrium, hyperplasia, cystic	8 (17%)	10 (21%)	8 (17%)	15 (31%)	12 (25%)
Horn, dilatation	2 (4%)	1 (2%)	1 (2%)		1 (2%)
Vagina	(1)	(4)	(1)	(4)	(5)
Dilatation					1 (20%)
Inflammation		1 (25%)		2 (50%)	
Mucocyte, metaplasia	1 (100%)	4 (100%)	1 (100%)	2 (50%)	3 (6%)
Hematopoietic System					
Bone marrow	(48)	(48)	(48)	(47)	(48)
Atrophy	3 (6%)	4 (8%)	3 (6%)	1 (2%)	3 (6%)
Hyperplasia		1 (2%)	1 (2%)	2 (4%)	3 (6%)
Thrombosis			1 (2%)		
Myeloid cell, hyperplasia				1 (2%)	1 (2%)
Lymph node	(7)	(9)	(10)	(6)	(9)
Degeneration, cystic				1 (17%)	
Axillary, infiltration cellular, plasma cell					1 (11%)
Iliac, degeneration, cystic					1 (11%)
Lumbar, degeneration, cystic		2 (22%)			1 (11%)
Lumbar, medulla sinus, dilatation					1 (11%)
Mediastinal, hemorrhage					1 (11%)
Mediastinal, hyperplasia, lymphoid			1 (10%)		1 (11%)
Mediastinal, inflammation				1 (17%)	
Mediastinal, pigmentation	1 (14%)				1 (11%)
Mediastinal, medulla sinus, dilatation		1 (11%)			1 (11%)
Medula, pancreatic sinus, dilatation	1 (14%)				
Pancreatic, necrosis					1 (11%)
Renal, degeneration, cystic					1 (11%)
Renal, hemorrhage	1 (14%)	1 (11%)	1 (10%)		
Renal, hyperplasia, lymphoid	1 (14%)	1 (11%)			1 (11%)
Lymph node, mandibular	(48)	(48)	(48)	(48)	(48)
Atrophy, lymphocyte	1 (2%)				
Degeneration, cystic	7 (15%)	6 (13%)	5 (10%)	2 (4%)	4 (8%)
Hyperplasia, lymphoid	1 (2%)		2 (4%)		1 (2%)
Infiltration cellular, plasma cell	4 (8%)	7 (15%)	2 (4%)	6 (13%)	8 (17%)
Medulla, sinus, dilatation	1 (2%)				1 (2%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Hematopoietic System (continued)					
Lymph node, mesenteric	(48)	(47)	(48)	(47)	(48)
Degeneration, cystic			1 (2%)	3 (6%)	1 (2%)
Hemorrhage	1 (2%)	1 (2%)	1 (2%)		1 (2%)
Hyperplasia, lymphoid	2 (4%)	1 (2%)	1 (2%)	1 (2%)	
Infiltration cellular, histiocyte		1 (2%)			
Infiltration cellular, mast cell		1 (2%)			
Lymphocyte, atrophy	1 (2%)	2 (4%)			1 (2%)
Medulla, sinus, dilatation			1 (2%)		
Spleen	(48)	(48)	(48)	(48)	(48)
Accessory spleen			1 (2%)		
Hematopoietic cell proliferation	8 (17%)	10 (21%)	7 (15%)	7 (15%)	15 (31%)
Hyperplasia, lymphoid	1 (2%)				
Infarct					3 (6%)
Pigmentation	3 (6%)	6 (13%)	4 (8%)	6 (13%)	5 (10%)
Capsule, proliferation connective tissue				1 (2%)	
Red pulp, atrophy	1 (2%)	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Red pulp, hyperplasia				2 (4%)	
Thymus	(47)	(47)	(45)	(45)	(46)
Atrophy	44 (94%)	44 (94%)	40 (89%)	39 (87%)	40 (87%)
Cyst	4 (9%)	1 (2%)	3 (7%)	1 (2%)	1 (2%)
Hemorrhage	1 (2%)				
Infiltration cellular, polymorphonuclear		1 (2%)			
Integumentary System					
Mammary gland	(48)	(48)	(46)	(47)	(48)
Galactocele	6 (13%)	9 (19%)	5 (11%)	2 (4%)	6 (13%)
Inflammation			1 (2%)		1 (2%)
Lactation	23 (48%)	30 (63%)	27 (59%)	22 (47%)	23 (48%)
Alveolus, hyperplasia	37 (77%)	37 (77%)	33 (72%)	29 (62%)	26 (54%)
Skin	(48)	(48)	(48)	(48)	(48)
Fibrosis			1 (2%)		
Inflammation	1 (2%)	2 (4%)	3 (6%)	1 (2%)	
Ulcer	2 (4%)		1 (2%)	1 (2%)	
Epidermis, hyperplasia	1 (2%)			1 (2%)	
Epidermis, necrosis		2 (4%)			
Tail, hyperkeratosis	1 (2%)				
Musculoskeletal System					
Bone	(0)	(0)	(0)	(0)	(1)
Bone, femur	(48)	(48)	(48)	(48)	(48)
Fibrous osteodystrophy	1 (2%)				
Osteopetrosis	5 (10%)	2 (4%)	2 (4%)	6 (13%)	3 (6%)
Skeletal muscle	(48)	(48)	(48)	(48)	(48)
Nervous System					
Brain, brain stem	(48)	(48)	(48)	(48)	(48)
Gliosis, focal	1 (2%)				
Hemorrhage			1 (2%)		
Infiltration cellular, mononuclear cell				1 (2%)	
Hypothalamus, compression	10 (21%)	13 (27%)	10 (21%)	8 (17%)	8 (17%)
Brain, cerebellum	(48)	(48)	(48)	(48)	(48)
Brain, cerebrum	(48)	(48)	(48)	(48)	(48)
Compression					1 (2%)
Gliosis, focal		1 (2%)			1 (2%)
Ventricle, dilatation	1 (2%)	2 (4%)		1 (2%)	2 (4%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Nervous System (continued)					
Peripheral nerve, sciatic	(48)	(48)	(48)	(48)	(48)
Axon, degeneration	4 (8%)	3 (6%)	1 (2%)	4 (8%)	19 (40%)
Spinal cord	(0)	(0)	(0)	(1)	(0)
Keratin cyst				1 (100%)	
Spinal cord, cervical	(48)	(48)	(48)	(48)	(48)
Cyst					1 (2%)
Gliosis, focal					1 (2%)
Hemorrhage				2 (4%)	
Axon, degeneration	22 (46%)	18 (38%)	15 (31%)	16 (33%)	10 (21%)
Nerve, degeneration	2 (4%)			1 (2%)	
Spinal cord, lumbar	(48)	(48)	(48)	(48)	(48)
Gliosis, focal			1 (2%)		2 (4%)
Axon, degeneration	1 (2%)	2 (4%)	4 (8%)	1 (2%)	2 (4%)
Nerve, degeneration	16 (33%)	21 (44%)	17 (35%)	16 (33%)	15 (31%)
Spinal cord, thoracic	(48)	(48)	(48)	(48)	(48)
Gliosis, focal					1 (2%)
Axon, degeneration	18 (38%)	22 (46%)	20 (42%)	15 (31%)	16 (33%)
Nerve, degeneration	1 (2%)	1 (2%)			1 (2%)
Respiratory System					
Lung	(48)	(48)	(48)	(48)	(48)
Foreign body				1 (2%)	
Granuloma	6 (13%)	5 (10%)	9 (19%)	4 (8%)	4 (8%)
Inflammation	1 (2%)	1 (2%)	4 (8%)	3 (6%)	4 (8%)
Alveolar epithelium, hyperplasia	1 (2%)			1 (2%)	2 (4%)
Alveolus, infiltration cellular, histiocyte	13 (27%)	7 (15%)	10 (21%)	10 (21%)	8 (17%)
Nose	(47)	(48)	(48)	(48)	(48)
Foreign body				2 (4%)	
Fungus					1 (2%)
Inflammation	5 (11%)	6 (13%)	7 (15%)	8 (17%)	8 (17%)
Osteopetrosis	3 (6%)	2 (4%)	1 (2%)	4 (8%)	2 (4%)
Special Senses System					
Eye	(45)	(48)	(47)	(45)	(46)
Cataract		2 (4%)	3 (6%)	1 (2%)	1 (2%)
Phthisis bulbi			1 (2%)	1 (2%)	
Retina, degeneration	14 (31%)	16 (33%)	16 (34%)	21 (47%)	23 (50%)
Sclera, metaplasia, osseous	2 (4%)		1 (2%)		1 (2%)
Harderian gland	(48)	(48)	(48)	(48)	(48)
Atrophy	1 (2%)	1 (2%)			
Infiltration cellular, lymphocyte	14 (29%)	17 (35%)	17 (35%)	17 (35%)	17 (35%)
Inflammation	1 (2%)	1 (2%)		1 (2%)	1 (2%)
Lacrimal gland	(0)	(1)	(1)	(0)	(1)
Metaplasia		1 (100%)	1 (100%)		
Zymbal's gland	(0)	(1)	(0)	(0)	(3)
Hyperplasia					1 (33%)
Urinary System					
Kidney	(48)	(48)	(48)	(48)	(48)
Accumulation, hyaline droplet				1 (2%)	
Cyst					2 (4%)
Hydronephrosis		1 (2%)			1 (2%)
Infarct		1 (2%)			
Infiltration cellular, lymphocyte		1 (2%)			
Mineralization	21 (44%)	21 (44%)	22 (46%)	24 (50%)	24 (50%)
Nephropathy	44 (92%)	34 (71%)	32 (67%)	33 (69%)	26 (54%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Urinary System (continued)					
Kidney					
Pigmentation				2 (4%)	
Cortex, inflammation, chronic				1 (2%)	
Renal tubule, necrosis			1 (2%)		
Urinary Bladder	(48)	(48)	(48)	(48)	(47)
Dilatation	1 (2%)	1 (2%)		3 (6%)	1 (2%)
Infiltration cellular, lymphocyte	2 (4%)		1 (2%)		1 (2%)

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

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF ACRYLAMIDE

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Drinking Water Study of Acrylamide[a]

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Disposition Summary					
Animals initially in study	48	48	48	48	48
Early deaths					
Moribund sacrifice	2	5	4	7	9
Natural deaths	6	2	3	3	7
Survivors					
Moribund sacrifice	1		4		3
Natural deaths		2			1
Terminal sacrifice	39	39	37	38	28
Animals examined microscopically	48	48	48	48	48
Alimentary System					
Gallbladder	(43)	(42)	(45)	(45)	(41)
Lymphoma malignant	1 (2%)				
Intestine large, cecum	(43)	(44)	(45)	(46)	(40)
Lymphoma malignant				1 (2%)	1 (3%)
Intestine small, duodenum	(43)	(44)	(45)	(45)	(40)
Adenoma		1 (2%)	1 (2%)		
Intestine small, ileum	(43)	(44)	(45)	(46)	(41)
Lymphoma malignant					2 (5%)
Intestine small, jejunum	(44)	(44)	(45)	(46)	(42)
Adenoma					1 (2%)
Hemangiosarcoma					1 (2%)
Lymphoma malignant				2 (4%)	3 (7%)
Liver	(46)	(48)	(47)	(46)	(47)
Hemangiosarcoma		2 (4%)		1 (2%)	
Hepatocellular adenoma	5 (11%)	8 (17%)	7 (15%)	3 (7%)	5 (11%)
Hepatocellular adenoma, multiple			3 (6%)	1 (2%)	1 (2%)
Hepatocellular carcinoma	5 (11%)	1 (2%)	4 (9%)	6 (13%)	5 (11%)
Hepatocellular carcinoma, multiple	1 (2%)			2 (4%)	2 (4%)
Hepatocholangiocarcinoma	1 (2%)				
Histiocytic sarcoma	2 (4%)	1 (2%)	1 (2%)	1 (2%)	4 (9%)
Ito cell tumor benign		1 (2%)			
Leukemia		1 (2%)			1 (2%)
Lymphoma malignant	1 (2%)				4 (9%)
Squamous cell carcinoma, metastatic, stomach, forestomach					1 (2%)
Mesentery	(0)	(1)	(0)	(1)	(0)
Oral mucosa	(1)	(0)	(0)	(0)	(0)
Squamous cell carcinoma	1 (100%)				
Pancreas	(45)	(46)	(47)	(47)	(46)
Lymphoma malignant	2 (4%)				1 (2%)
Sarcoma					1 (2%)
Salivary glands	(45)	(46)	(47)	(47)	(45)
Lymphoma malignant	1 (2%)				
Stomach, forestomach	(46)	(45)	(46)	(47)	(44)
Sarcoma					1 (2%)
Squamous cell carcinoma				1 (2%)	2 (5%)
Squamous cell papilloma		2 (4%)	2 (4%)	6 (13%)	5 (11%)
Squamous cell papilloma, multiple					1 (2%)
Stomach, glandular	(44)	(45)	(46)	(46)	(41)
Adenoma				1 (2%)	
Tongue	(0)	(0)	(0)	(0)	(1)
Squamous cell papilloma					1 (100%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Cardiovascular System					
Blood vessel	(47)	(47)	(46)	(47)	(48)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)				
Lymphoma malignant	1 (2%)				3 (6%)
Heart	(47)	(47)	(47)	(47)	(48)
Heatocholangiocarcinoma, metastatic, liver	1 (2%)				
Leukemia					1 (2%)
Lymphoma malignant	1 (2%)				3 (6%)
Endocrine System					
Adrenal cortex	(45)	(46)	(47)	(47)	(44)
Lymphoma malignant	1 (2%)				1 (2%)
Adrenal medulla	(44)	(44)	(46)	(47)	(44)
Pheochromocytoma benign		1 (2%)		1 (2%)	
Pheochromocytoma malignant				1 (2%)	
Islets, pancreatic	(46)	(46)	(47)	(47)	(46)
Lymphoma malignant	1 (2%)				
Parathyroid gland	(43)	(44)	(44)	(44)	(41)
Pituitary gland	(44)	(47)	(46)	(45)	(42)
Lymphoma malignant					2 (5%)
Thyroid gland	(46)	(46)	(46)	(47)	(47)
Follicular cell, adenoma	1 (2%)				
General Body System					
None					
Genital System					
Epididymis	(46)	(46)	(47)	(47)	(44)
Histiocytic sarcoma					1 (2%)
Lymphoma malignant	2 (4%)				
Penis	(0)	(0)	(1)	(0)	(1)
Preputial gland	(44)	(46)	(47)	(47)	(46)
Histiocytic sarcoma	1 (2%)				
Squamous cell carcinoma					1 (2%)
Squamous cell papilloma					1 (2%)
Prostate	(45)	(45)	(47)	(47)	(44)
Seminal vesicle	(45)	(46)	(47)	(47)	(44)
Lymphoma malignant					1 (2%)
Testes	(45)	(44)	(46)	(47)	(43)
Hematopoietic System					
Bone marrow	(46)	(47)	(47)	(47)	(44)
Hemangiosarcoma		1 (2%)			1 (2%)
Histiocytic sarcoma	2 (4%)	1 (2%)			2 (5%)
Leukemia					1 (2%)
Lymphoma malignant					1 (2%)
Lymph node	(3)	(2)	(4)	(4)	(11)
Axillary, lymphoma malignant					1 (9%)
Inguinal, histiocytic sarcoma					2 (18%)
Inguinal, lymphoma malignant					1 (9%)
Lumbar, histiocytic sarcoma					2 (18%)
Lumbar, lymphoma malignant	1 (33%)		1 (25%)		2 (18%)
Mediastinal, hepatocholangiocarcinoma, metastatic, liver	1 (33%)				
Mediastinal, lymphoma malignant	1 (33%)				3 (27%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Hematopoietic System (continued)					
Lymph node (continued)					
Pancreatic, histiocytic sarcoma					3 (27%)
Pancreatic, lymphoma malignant	1 (33%)	1 (50%)		2 (50%)	1 (9%)
Pancreatic, sarcoma					1 (9%)
Renal, histiocytic sarcoma					1 (9%)
Renal, lymphoma malignant	1 (33%)		1 (25%)	2 (50%)	2 (18%)
Lymph node, mandibular	(47)	(45)	(47)	(47)	(46)
Histiocytic sarcoma	1 (2%)		1 (2%)		3 (7%)
Lymphoma malignant	2 (4%)		1 (2%)	1 (2%)	3 (7%)
Lymph node, mesenteric	(44)	(45)	(47)	(46)	(45)
Hemangiosarcoma					1 (2%)
Histiocytic sarcoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)	4 (9%)
Leukemia					1 (2%)
Lymphoma malignant	2 (5%)	1 (2%)	1 (2%)	2 (4%)	7 (16%)
Spleen	(45)	(47)	(46)	(47)	(45)
Hemangiosarcoma		3 (6%)	2 (4%)		
Histiocytic sarcoma	2 (4%)	1 (2%)	1 (2%)		3 (7%)
Leukemia		1 (2%)			1 (2%)
Lymphoma malignant	2 (4%)	1 (2%)	1 (2%)	3 (6%)	4 (9%)
Thymus	(43)	(42)	(41)	(42)	(40)
Histiocytic sarcoma					1 (3%)
Lymphoma malignant	4 (9%)				5 (13%)
Integumentary System					
Skin	(47)	(47)	(47)	(47)	(46)
Basosquamous tumor benign					2 (4%)
Squamous cell carcinoma				1 (2%)	
Squamous cell papilloma	1 (2%)			2 (4%)	3 (7%)
Subcutaneous tissue, fibroma	1 (2%)	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Subcutaneous tissue, fibrosarcoma	2 (4%)	2 (4%)	2 (4%)	1 (2%)	2 (4%)
Subcutaneous tissue, fibrous histiocytoma			1 (2%)		1 (2%)
Subcutaneous tissue, lipoma					1 (2%)
Subcutaneous tissue, liposarcoma		1 (2%)			
Subcutaneous tissue, sarcoma	2 (4%)	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Subcutaneous tissue, schwannoma malignant				2 (4%)	1 (2%)
Musculoskeletal System					
Bone, femur	(48)	(48)	(48)	(48)	(48)
Skeletal muscle	(45)	(46)	(47)	(47)	(44)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)				
Nervous System					
Brain, brain stem	(46)	(46)	(47)	(47)	(45)
Leukemia	1 (2%)				
Lymphoma malignant					1 (2%)
Brain, cerebellum	(46)	(46)	(47)	(47)	(45)
Leukemia	1 (2%)				
Lymphoma malignant					1 (2%)
Brain, cerebrum	(46)	(46)	(47)	(47)	(45)
Leukemia	1 (2%)				
Peripheral nerve, sciatic	(46)	(46)	(47)	(47)	(45)
Spinal cord, cervical	(46)	(45)	(47)	(46)	(46)
Meninges, lymphoma malignant					1 (2%)
Spinal cord, lumbar	(46)	(45)	(47)	(47)	(46)
Meninges, lymphoma malignant					1 (2%)
Spinal cord, thoracic	(46)	(45)	(47)	(47)	(47)
Meninges, lymphoma malignant					1 (2%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Respiratory System					
Lung	(47)	(46)	(47)	(45)	(48)
Alveolar/bronchiolar adenoma	3 (6%)	6 (13%)	12 (26%)	7 (16%)	13 (27%)
Alveolar/bronchiolar adenoma, multiple	2 (4%)		1 (2%)	3 (7%)	6 (13%)
Alveolar/bronchiolar carcinoma	2 (4%)		1 (2%)	1 (2%)	4 (8%)
Fibrosarcoma, metastatic, skin		1 (2%)			
Hepatocellular carcinoma, metastatic, liver			1 (2%)	1 (2%)	
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)				
Histiocytic sarcoma	2 (4%)		1 (2%)		2 (4%)
Leukemia		1 (2%)			1 (2%)
Liposarcoma, metastatic, skin		1 (2%)			
Lymphoma malignant	4 (9%)				3 (6%)
Nose	(45)	(45)	(47)	(47)	(46)
Lymphoma malignant					1 (2%)
Special Senses System					
Eye	(44)	(44)	(45)	(44)	(41)
Harderian gland	(46)	(46)	(47)	(47)	(47)
Adenocarcinoma				1 (2%)	1 (2%)
Adenoma	2 (4%)	13 (28%)	21 (45%)	22 (47%)	15 (32%)
Histiocytic sarcoma					1 (2%)
Lymphoma malignant					1 (2%)
Bilateral, adenoma			6 (13%)	14 (30%)	24 (51%)
Urinary System					
Kidney	(45)	(46)	(47)	(47)	(44)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)				
Histiocytic sarcoma	1 (2%)				1 (2%)
Lymphoma malignant	1 (2%)				3 (7%)
Renal tubule, carcinoma		1 (2%)			
Urinary bladder	(46)	(47)	(46)	(45)	(43)
Lymphoma malignant					1 (2%)
Systemic Lesions					
Multiple organs	(48)[b]	(48)[b]	(48)[b]	(48)[b]	(48)[b]
Histiocytic sarcoma	2 (4%)	1 (2%)	1 (2%)	2 (4%)	4 (8%)
Leukemia	1 (2%)	1 (2%)			1 (2%)
Lymphoma malignant	4 (8%)	1 (2%)	1 (2%)	4 (8%)	9 (19%)
Neoplasm Summary					
Total animals with primary neoplasms[c]	25	35	40	44	46
Total primary neoplasms	36	48	67	86	120
Total animals with benign neoplasms	13	28	39	41	45
Total benign neoplasms	15	33	54	62	80
Total animals with malignant neoplasms	19	14	13	20	29
Total malignant neoplasms	21	15	13	24	40
Total animals with metastatic neoplasms	1	2	1	1	1
Total metastatic neoplasms	6	2	1	1	1

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

TABLE C2
Statistical Analysis of Neoplasms in Male Mice
in the 2-Year Drinking Water Study of Acrylamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Harderian Gland: Adenoma					
Overall rate[a]	2/46 (4%)	13/46 (28%)	27/47 (57%)	36/47 (77%)	39/47 (83%)
Adjusted rate[b]	4.8%	30.1%	60.1%	79.9%	87.7%
Terminal rate[c]	2/39 (5%)	11/39 (28%)	22/37 (60%)	30/38 (79%)	25/28 (89%)
First incidence (days)[d]	732 (T)	610	422	551	456
Poly-3 test[e]	P<0.001	P=0.002	P<0.001	P<0.001	P<0.001
Harderian Gland: Adenocarcinoma					
Overall rate	0/46 (0%)	0/46 (0%)	0/47 (0%)	1/47 (2%)	1/47 (2%)
Adjusted rate	0%	0%	0%	2.3%	2.6%
Terminal rate	0/39 (0%)	0/39 (0%)	0/37 (0%)	1/38 (2.6%)	1/28 (3.6%)
First incidence (days)	-	-	-	732 (T)	732 (T)
Poly-3 test	P=0.138	-	-	P=0.508	P=0.487
Harderian Gland: Adenoma or Adenocarcinoma					
Overall rate	2/46 (4%)	13/46 (28%)	27/47 (57%)	37/47 (79%)	39/47 (83%)
Adjusted rate	4.8%	30.1%	60.1%	82.1%	87.7%
Terminal rate	2/39 (5%)	11/39 (28%)	22/37 (60%)	31/38 (82%)	25/28 (89%)
First incidence (days)	732 (T)	610	422	551	456
Poly-3 test	P<0.001	P=0.002	P<0.001	P<0.001	P<0.001
Liver: Hepatocellular Adenoma					
Overall rate	5/46 (11%)	8/48 (17%)	10/47 (21%)	4/46 (9%)	6/47 (13%)
Adjusted rate	12.1%	18.2%	22.9%	9.3%	15.5%
Terminal rate	5/39 (13%)	8/39 (21%)	8/37 (22%)	3/38 (8%)	5/28 (18%)
First incidence (days)	732 (T)	732 (T)	621	659	653
Poly-3 test	P=0.430N	P=0.313	P=0.151	P=0.477N	P=0.453
Liver: Hepatocellular Carcinoma					
Overall rate	6/46 (13%)	1/48 (2%)	4/47 (9%)	8/46 (17%)	7/47 (15%)
Adjusted rate	14.2%	2.3%	9.2%	18.1%	17.6%
Terminal rate	5/39 (13%)	1/39 (3%)	2/37 (5%)	4/38 (11%)	3/28 (11%)
First incidence (days)	366	732 (T)	683	551	590
Poly-3 test	P=0.076	P=0.050N	P=0.354N	P=0.420	P=0.453
Liver: Hepatocellular Adenoma or Carcinoma					
Overall rate	10/46 (22%)	8/48 (17%)	14/47 (30%)	11/46 (24%)	12/47 (26%)
Adjusted rate	23.6%	18.2%	31.9%	24.9%	29.9%
Terminal rate	9/39 (23%)	8/39 (21%)	10/37 (27%)	7/38 (18%)	7/28 (25%)
First incidence (days)	366	732 (T)	621	551	590
Poly-3 test	P=0.243	P=0.363N	P=0.269	P=0.545	P=0.347
Lung: Alveolar/bronchiolar Adenoma					
Overall rate	5/47 (11%)	6/46 (13%)	13/47 (28%)	10/45 (22%)	19/48 (40%)
Adjusted rate	11.9%	13.8%	29.9%	23.6%	47.3%
Terminal rate	5/39 (13%)	6/39 (15%)	12/37 (32%)	9/38 (24%)	14/28 (50%)
First incidence (days)	732 (T)	732 (T)	636	674	512
Poly-3 test	P<0.001	P=0.526	P=0.036	P=0.133	P<0.001
Lung: Alveolar/bronchiolar Carcinoma					
Overall rate	2/47 (4%)	0/46 (0%)	1/47 (2%)	1/45 (2%)	4/48 (8%)
Adjusted rate	4.8%	0%	2.3%	2.4%	10.2%
Terminal rate	2/39 (5%)	0/39 (0%)	1/37 (3%)	0/38 (0%)	3/28 (11%)
First incidence (days)	732 (T)	-	732 (T)	674	691
Poly-3 test	P=0.056	P=0.229N	P=0.489N	P=0.495N	P=0.305

TABLE C2
Statistical Analysis of Neoplasms in Male Mice
in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Lung: Alveolar/bronchiolar Adenoma or Carcinoma					
Overall rate	6/47 (13%)	6/46 (13%)	14/47 (30%)	10/45 (22%)	20/48 (42%)
Adjusted rate	14.3%	13.8%	32.2%	23.6%	49.8%
Terminal rate	6/39 (15%)	6/39 (15%)	13/37 (35%)	9/38 (24%)	15/28 (54%)
First incidence (days)	732 (T)	732 (T)	636	674	512
Poly-3 test	P<0.001	P=0.595N	P=0.043	P=0.211	P<0.001
Stomach (Forestomach): Squamous Cell Papilloma					
Overall rate	0/46 (0%)	2/45 (4%)	2/46 (4%)	6/47 (13%)	6/44 (14%)
Adjusted rate	0%	4.7%	4.7%	13.7%	16.5%
Terminal rate	0/39 (0%)	2/39 (5%)	2/37 (5%)	5/38 (13%)	5/28 (18%)
First incidence (days)	-	732 (T)	732 (T)	502	729
Poly-3 test	P=0.002	P=0.243	P=0.242	P=0.018	P=0.009
Stomach (Forestomach): Squamous Cell Carcinoma					
Overall rate	0/46 (0%)	0/45 (0%)	0/46 (0%)	1/47 (2%)	2/44 (5%)
Adjusted rate	0%	0%	0%	2.3%	5.5%
Terminal rate	0/39 (0%)	0/39 (0%)	0/37 (0%)	1/38 (3%)	2/28 (7%)
First incidence (days)	-	-	-	732 (T)	732 (T)
Poly-3 test	P=0.024	-	-	P=0.508	P=0.209
Stomach (Forestomach): Squamous Cell Papilloma or Squamous Cell Carcinoma					
Overall rate	0/46 (0%)	2/45 (4%)	2/46 (4%)	7/47 (15%)	8/44 (18%)
Adjusted rate	0%	4.7%	4.7%	16.0%	21.9%
Terminal rate	0/39 (0%)	2/39 (5%)	2/37 (5%)	6/38 (16%)	7/28 (25%)
First incidence (days)	-	732 (T)	732 (T)	502	729
Poly-3 test	P<0.001	P=0.243	P=0.242	P=0.009	P=0.002
Stomach (Forestomach): Sarcoma, or Squamous Cell Papilloma or Carcinoma					
Overall rate	0/46 (0%)	2/45 (4%)	2/46 (4%)	7/47 (15%)	9/44 (21%)
Adjusted rate	0%	4.7%	4.7%	16.0%	24.4%
Terminal rate	0/39 (0%)	2/39 (5%)	2/37 (5%)	6/38 (16%)	7/28 (25%)
First incidence (days)	-	732 (T)	732 (T)	502	631
Poly-3 test	P<.001	P=0.243	P=0.242	P=0.009	P<.001
Skin: Squamous Cell Papilloma					
Overall rate	1/47 (2%)	0/47 (0%)	0/47 (0%)	2/47 (4%)	3/46 (7%)
Adjusted rate	2.4%	0%	0%	4.6%	7.9%
Terminal rate	1/39 (3%)	0/39 (0%)	0/37 (0%)	2/38 (5%)	2/28 (7%)
First incidence (days)	732 (T)	-	-	732 (T)	631
Poly-3 test	P=0.029	P=0.493N	P=0.494N	P=0.511	P=0.271
Skin: Squamous Cell Carcinoma					
Overall rate	0/47 (0%)	0/47 (0%)	0/47 (0%)	1/47 (2%)	0/46 (0%)
Adjusted rate	0%	0%	0%	2.3%	0%
Terminal rate	0/39 (0%)	0/39 (0%)	0/37 (0%)	1/38 (3%)	0/28 (0%)
First incidence (days)	-	-	-	732 (T)	-
Poly-3 test	P=0.578	-	-	P=0.506	-
Skin: Squamous Cell Papilloma or Carcinoma					
Overall rate[a]	1/47 (2%)	0/47 (0%)	0/47 (0%)	2/47 (4%)	3/46 (7%)
Adjusted rate[b]	2.4%	0%	0%	4.6%	7.9%
Terminal rate[c]	1/39 (3%)	0/39 (0%)	0/37 (0%)	2/38 (5%)	2/28 (7%)
First incidence (days)[d]	732 (T)	-	-	732 (T)	631
Poly-3 test[e]	P=0.029	P=0.493N	P=0.494N	P=0.511	P=0.271

TABLE C2
Statistical Analysis of Neoplasms in Male Mice
in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Skin (Subcutaneous Tissue): Fibrosarcoma, Fibrous Histiocytoma, or Sarcoma					
Overall rate	4/47 (9%)	3/47 (6%)	4/47 (9%)	2/47 (4%)	4/46 (9%)
Adjusted rate	9.6%	6.7%	9.0%	4.6%	10.3%
Terminal rate	4/39 (10%)	0/39 (0%)	1/37 (3%)	1/38 (3%)	1/28 (4%)
First incidence (days)	732 (T)	450	422	502	512
Poly-3 test	P=0.513	P=0.460N	P=0.613N	P=0.315N	P=0.602
Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, Fibrous Histiocytoma, or Sarcoma					
Overall rate	5/47 (11%)	4/47 (9%)	5/47 (11%)	4/47 (9%)	5/46 (11%)
Adjusted rate	11.9%	8.9%	11.3%	9.1%	12.9%
Terminal rate	5/39 (13%)	1/39 (3%)	2/37 (5%)	3/38 (8%)	2/28 (7%)
First incidence (days)	732 (T)	450	422	502	512
Poly-3 test	P=0.456	P=0.454N	P=0.595N	P=0.471N	P=0.583
Skin: All Morphologies					
Overall rate	5/47 (11%)	5/47 (11%)	5/47 (11%)	8/47 (17%)	10/46 (22%)
Adjusted rate	11.9%	11.0%	11.3%	18.1%	25.5%
Terminal rate	5/39 (13%)	1/39 (3%)	2/37 (5%)	6/38 (16%)	6/28 (21%)
First incidence (days)	732 (T)	450	422	502	512
Poly-3 test	P=0.024	P=0.577N	P=0.595N	P=0.312	P=0.098
Spleen: Hemangiosarcoma					
Overall rate	0/45 (0%)	3/47 (6%)	2/46 (4%)	0/47 (0%)	0/45 (0%)
Adjusted rate	0%	6.8%	4.6%	0%	0%
Terminal rate	0/39 (0%)	3/39 (8%)	2/37 (5%)	0/38 (0%)	0/28 (0%)
First incidence (days)	-	732 (T)	732 (T)	-	-
Poly-3 test	P=0.179N	P=0.129	P=0.246	-	-
All Organs: Leukemia					
Overall rate	1/48 (2%)	1/48 (2%)	0/48 (0%)	0/48 (0%)	1/48 (2%)
Adjusted rate	2.4%	2.3%	0%	0.0%	2.6%
Terminal rate	0/39 (0%)	0/39 (0%)	0/37 (0%)	0/38 (0%)	0/28 (0%)
First incidence (days)	631	670	-	-	715
Poly-3 test	P=0.644	P=0.750N	P=0.495N	P=0.493N	P=0.743
All Organs: Malignant Lymphoma					
Overall rate	4/48 (8%)	1/48 (2%)	1/48 (2%)	4/48 (8%)	9/48 (19%)
Adjusted rate	9.3%	2.3%	2.3%	9.1%	21.5%
Terminal rate	2/39 (5%)	1/39 (3%)	1/37 (3%)	3/38 (8%)	3/28 (11%)
First incidence (days)	523	732 (T)	732 (T)	703	456
Poly-3 test	P=0.002	P=0.171N	P=0.175N	P=0.631N	P=0.105
All Organs: Histiocytic Sarcoma					
Overall rate	2/48 (4%)	1/48 (2%)	1/48 (2%)	2/48 (4%)	4/48 (8%)
Adjusted rate	4.7%	2.3%	2.3%	4.5%	10.1%
Terminal rate	1/39 (3%)	0/39 (0%)	0/37 (0%)	0/38 (0%)	1/28 (4%)
First incidence (days)	631	595	621	603	600
Poly-3 test	P=0.090	P=0.482N	P=0.488N	P=0.675N	P=0.309
All Organs: Hemangiosarcoma or Hemangioma					
Overall rate	0/48 (0%)	5/48 (10%)	2/48 (4%)	1/48 (2%)	3/48 (6%)
Adjusted rate	0%	11.3%	4.6%	2.3%	7.6%
Terminal rate	0/39 (0%)	4/39 (10%)	2/37 (5%)	0/38 (0%)	2/28 (7%)
First incidence (days)	-	707	732 (T)	621	579
Poly-3 test	P=0.394	P=0.035	P=0.245	P=0.511	P=0.108

TABLE C2
Statistical Analysis of Neoplasms in Male Mice
in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
All Organs: Fibrous Histiocytoma					
Overall rate	0/48 (0%)	0/48 (0%)	1/48 (2%)	0/48 (0%)	1/48 (2%)
Adjusted rate	0%	0%	2.3%	0%	2.6%
Terminal rate	0/39 (0%)	0/39 (0%)	0/37 (0%)	0/38 (0%)	0/28 (0%)
First incidence (days)	-	-	687	-	723
Poly-3 test	P=0.288	-	P=0.508	-	P=0.486
All Organs: Benign Tumors					
Overall rate	13/48 (27%)	28/48 (58%)	39/48 (81%)	41/48 (85%)	45/48 (94%)
Adjusted rate	31.1%	62.9%	84.8%	88.6%	98.0%
Terminal rate	13/39 (33%)	26/39 (67%)	31/37 (84%)	34/38 (90%)	28/28 (100%)
First incidence (days)	732 (T)	610	422	502	456
Poly-3 test	P<0.001	P=0.002	P<0.001	P<0.001	P<0.001
All Organs: Malignant Tumors					
Overall rate	19/48 (40%)	14/48 (29%)	13/48 (27%)	20/48 (42%)	29/48 (60%)
Adjusted rate	42.1%	29.9%	28.8%	42.4%	64.8%
Terminal rate	13/39 (33%)	7/39 (18%)	7/37 (19%)	11/38 (29%)	13/28 (46%)
First incidence (days)	366	450	422	502	456
Poly-3 test	P<0.001	P=0.157N	P=0.135N	P=0.571	P=0.024
All Organs: Benign or Malignant Tumors					
Overall rate	25/48 (52%)	35/48 (73%)	40/48 (83%)	44/48 (92%)	46/48 (96%)
Adjusted rate	55.4%	74.7%	86.5%	93.4%	100%
Terminal rate	19/39 (49%)	28/39 (72%)	31/37 (84%)	35/38 (92%)	28/28 (100%)
First incidence (days)	366	450	422	502	456
Poly-3 test	P<0.001	P=0.041	P<0.001	P<0.001	P<0.001

[a] Number of animals with neoplasm per number of animals examined microscopically.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[c] Observed incidence at the terminal sacrifice.
[d] T indicates terminal sacrifice.
[e] Beneath the 0 mM acrylamide are the p values associated with the trend test. Beneath the treated groups incidences are the p values corresponding to pairwise comparisons between the 0 mM acrylamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice. An N indicates a negative trend or decreased tumor incidence.

TABLE C3a
Historical Incidence of Harderian Gland Neoplasms in NCTR Control Male B6C3F1 Mice

Study (Report Date)	Route of Administration	Incidence in Controls		
		Adenoma	Adenocarcinoma	Adenoma or Adenocarcinoma
Chloral Hydrate (July 2001)	Gavage	4/48 (8.3%)	0/48 (0.0%)	4/48 (8.3%)
Chloral Hydrate (October 2002)	Gavage	5/47 (10.6%)	0/47 (0.0%)	5/47 (10.6%)
Doxylamine (April 1991)	Diet	1/48 (2.1%)	0/48 (0.0%)	1/48 (2.1%)
Fumonisin B_1 (March 1999)	Diet	1/46 (2.2%)	0/46 (0.0%)	1/46 (2.2%)
Pyrilamine (July 1991)	Diet	2/47 (4.3%)	0/47 (0.0%)	2/47 (4.3%)
Sulfamethazine (February 1988)	Diet	15/184 (8.2%)	0/184 (0.0%)	15/184 (8.2%)
Triprolidine (June 1991)	Diet	0/48 (0.0%)	0/48 (0.0%)	0/48 (0.0%)
Urethane and Ethanol (May 2003)	Drinking Water	3/47 (6.4%)	0/47 (0.0%)	3/47 (6.4%)
Total (%)		31/515 (6.0%)	0/515 (0.0%)	31/515 (6.0%)
Range		0.0%-10.6%	0.0%	0.0%-10.6%

TABLE C3b
Historical Incidence of Alveolar/Bronchiolar Neoplasms in NCTR Control Male B6C3F1 Mice

Study (Report Date)	Route of Administration	Incidence in Controls		
		Adenoma	Carcinoma	Adenoma or Carcinoma
Chloral Hydrate (July 2001)	Gavage	4/48 (8.3%)	4/48 (8.3%)	8/48 (16.7%)
Chloral Hydrate (October 2002)	Gavage	13/48 (27.1%)	2/48 (4.2%)	15/48 (31.3%)
Doxylamine (April 1991)	Diet	9/48 (18.8%)	0/48 (0.0%)	9/48 (18.8%)
Fumonisin B_1 (March 1999)	Diet	6/48 (12.5%)	0/48 (0.0%)	6/48 (12.5%)
Pyrilamine (July 1991)	Diet	5/47 (10.6%)	0/47 (0.0%)	5/47 (10.6%)
Sulfamethazine (February 1988)	Diet	25/186 (13.4%)	3/186 (1.6%)	28/186 (15.1%)
Triprolidine (June 1991)	Diet	9/48 (18.8%)	2/48 (4.2%)	11/48 (22.9%)
Urethane and Ethanol (May 2003)	Drinking Water	4/48 (8.3%)	1/48 (2.1%)	5/48 (10.4%)
Total (%)		75/521 (14.4%)	12/521 (2.3%)	87/521 (16.7%)
Range		8.3%-18.8%	0.0%-8.3%	10.4%-31.3%

TABLE C3c
Historical Incidence of Squamous Cell Papilloma or Carcinoma (Combined) of the Forestomach in NCTR Control Male B6C3F1 Mice

Study (Report Date)	Route of Administration	Incidence in Controls
Chloral Hydrate (July 2001)	Gavage	0/48 (0.0%)
Chloral Hydrate (October 2002)	Gavage	0/43 (0.0%)
Doxylamine (April 1991)	Diet	0/47 (0.0%)
Fumonisin B_1 (March 1999)	Diet	1/47 (2.1%)
Pyrilamine (July 1991)	Diet	0/46 (0.0%)
Sulfamethazine (February 1988)	Diet	1/179 (0.6%)
Triprolidine (June 1991)	Diet	0/48 (0.0%)
Urethane and Ethanol (May 2003)	Drinking Water	0/46 (0.0%)
Total (%)		2/504 (0.4%)
Range		0.0%-2.1%

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Drinking Water Study of Acrylamide[a]

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Disposition Summary					
Animals initially in study	48	48	48	48	48
Early deaths					
Moribund sacrifice	2	5	4	7	9
Natural deaths	6	2	3	3	7
Survivors					
Moribund sacrifice	1		4		3
Natural deaths		2			1
Terminal sacrifice	39	39	37	38	28
Animals examined microscopically	48	48	48	48	48
Alimentary System					
Gallbladder	(43)	(42)	(45)	(45)	(41)
Inflammation, suppurative				1 (2%)	
Inflammation, chronic active					1 (2%)
Lumen, dilatation	1 (2%)				
Intestine large, cecum	(43)	(44)	(45)	(46)	(40)
Hyperplasia, lymphoid	4 (9%)	6 (14%)	2 (4%)	1 (2%)	
Intestine small, duodenum	(43)	(44)	(45)	(45)	(40)
Hyperplasia, lymphoid			1 (2%)		
Intestine small, ileum	(43)	(44)	(45)	(46)	(41)
Angiectasis					1 (2%)
Hyperplasia, lymphoid	1 (2%)	1 (2%)			
Intestine small, jejunum	(44)	(44)	(45)	(46)	(42)
Hyperplasia, lymphoid		1 (2%)	1 (2%)	1 (2%)	2 (5%)
Inflammation, suppurative				1 (2%)	
Necrosis				1 (2%)	
Liver	(46)	(48)	(47)	(46)	(47)
Angiectasis				1 (2%)	
Basophilic focus		3 (6%)	1 (2%)	1 (2%)	1 (2%)
Basophilic focus, multiple				1 (2%)	
Hematopoietic cell proliferation			2 (4%)		2 (4%)
Infiltration cellular, lymphocyte	3 (7%)	1 (2%)		2 (4%)	
Inflammation, chronic			1 (2%)		
Inflammation, chronic active	2 (4%)				1 (2%)
Necrosis		2 (4%)	2 (4%)		
Thrombus	1 (2%)				
Vacuolization cytoplasmic				1 (2%)	
Mesentery	(0)	(1)	(0)	(1)	(0)
Fat, necrosis		1 (100%)		1 (100%)	
Oral mucosa	(1)	(0)	(0)	(0)	(0)
Pancreas	(45)	(46)	(47)	(47)	(46)
Edema	1 (2%)				
Infiltration cellular, lymphocyte	2 (4%)	2 (4%)	3 (6%)	3 (6%)	
Inflammation, chronic active					1 (2%)
Acinus, degeneration	3 (7%)	5 (11%)	4 (9%)	3 (6%)	2 (4%)
Duct, dilatation		1 (2%)	1 (2%)	1 (2%)	
Salivary glands	(45)	(46)	(47)	(47)	(45)
Infiltration cellular, lymphocyte	17 (38%)	20 (43%)	23 (49%)	18 (38%)	13 (29%)
Inflammation, chronic active					1 (2%)
Stomach, forestomach	(46)	(45)	(46)	(47)	(44)
Keratin cyst	2 (4%)	1 (2%)		5 (11%)	1 (2%)
Ulcer					1 (2%)
Epithelium, hyperplasia		1 (2%)	3 (7%)	3 (6%)	8 (18%)
Stomach, glandular	(44)	(45)	(46)	(46)	(41)
Inflammation, chronic active	1 (2%)				
Epithelium, hyperplasia	1 (2%)				
Tongue	(0)	(0)	(0)	(0)	(1)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Cardiovascular System					
Blood vessel	(47)	(47)	(46)	(47)	(48)
Heart	(47)	(47)	(47)	(47)	(48)
Cardiomyopathy				1 (2%)	
Polyarteritis			1 (2%)		
Endocrine System					
Adrenal cortex	(45)	(46)	(47)	(47)	(44)
Accessory adrenal cortical nodule	1 (2%)		1 (2%)		
Hyperplasia	1 (2%)	1 (2%)			
Hyperplasia, lymphoid	1 (2%)				
Hypertrophy	1 (2%)		1 (2%)	1 (2%)	1 (2%)
Metaplasia, osseous			1 (2%)		
Subcapsular, hyperplasia	35 (78%)	39 (85%)	41 (87%)	39 (83%)	31 (70%)
Adrenal Medulla	(44)	(44)	(46)	(47)	(44)
Hyperplasia					1 (2%)
Islets, pancreatic	(46)	(46)	(47)	(47)	(46)
Hyperplasia	1 (2%)	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Parathyroid gland	(43)	(44)	(44)	(44)	(41)
Cyst					1 (2%)
Pituitary gland	(44)	(47)	(46)	(45)	(42)
Pars distalis, cyst	1 (2%)	1 (2%)		1 (2%)	
Thyroid gland	(46)	(46)	(46)	(47)	(47)
Cyst	2 (4%)	1 (2%)			
Ectopic thymus			1 (2%)		
Infiltration cellular, lymphocyte	1 (2%)			1 (2%)	
Polyarteritis			1 (2%)		
Follicle, degeneration	5 (11%)	3 (7%)	5 (11%)	5 (11%)	5 (11%)
Follicular cell, hyperplasia					1 (2%)
General Body System					
None					
Genital System					
Epididymis	(46)	(46)	(47)	(47)	(44)
Angiectasis			1 (2%)		
Exfoliated germ cell					1 (2%)
Hypospermia	2 (4%)			3 (6%)	
Infiltration cellular, lymphocyte			1 (2%)		1 (2%)
Inflammation, suppurative			1 (2%)		
Inflammation, chronic active		1 (2%)			1 (2%)
Spermatocele			2 (4%)		1 (2%)
Penis	(0)	(0)	(1)	(0)	(1)
Inflammation, suppurative			1 (100%)		
Inflammation, chronic active					1 (100%)
Necrosis					1 (100%)
Ulcer					1 (100%)
Epithelium, hyperplasia			1 (100%)		
Preputial gland	(44)	(46)	(47)	(47)	(46)
Angiectasis					1 (2%)
Cyst	4 (9%)	3 (7%)	6 (13%)	4 (9%)	2 (4%)
Degeneration	9 (20%)	8 (17%)	9 (19%)	12 (26%)	15 (33%)
Dysplasia, focal					1 (2%)
Hemorrhage	1 (2%)				
Infiltration cellular, lymphocyte	6 (14%)	2 (4%)	4 (9%)	1 (2%)	3 (7%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Genital System (continued)					
Preputial gland					
Inflammation, suppurative	1 (2%)		1 (2%)	4 (9%)	8 (17%)
Inflammation, chronic				1 (2%)	
Inflammation, chronic active	2 (5%)	6 (13%)	2 (4%)	9 (19%)	7 (15%)
Duct, dilatation			1 (2%)	2 (4%)	2 (4%)
Prostate	(45)	(45)	(47)	(47)	(44)
Infiltration cellular, lymphocyte				1 (2%)	
Inflammation, chronic active					1 (2%)
Seminal vesicle	(45)	(46)	(47)	(47)	(44)
Inflammation, chronic active					1 (2%)
Lumen, dilatation		2 (4%)			
Testes	(45)	(44)	(46)	(47)	(43)
Mineralization		1 (2%)			
Spermatocele	1 (2%)				
Seminiferous tubule, degeneration	7 (16%)		4 (9%)	6 (13%)	8 (19%)
Hematopoietic System					
Bone marrow	(46)	(47)	(47)	(47)	(44)
Hyperplasia	3 (7%)	2 (4%)	3 (6%)	2 (4%)	5 (11%)
Thrombosis				2 (4%)	
Lymph node	(3)	(2)	(4)	(4)	(11)
Axillary, hyperplasia, lymphoid	1 (33%)		1 (25%)	1 (25%)	
Axillary, infiltration cellular, plasma cell			1 (25%)		
Fat, inguinal, inflammation, suppurative		1 (50%)			
Fat, inguinal, necrosis		1 (50%)			
Inguinal, hyperplasia, lymphoid	1 (33%)				
Lumbar, fibrosis					1 (9%)
Lumbar, hyperplasia, lymphoid				1 (25%)	
Lumbar, infiltration cellular, plasma cell			2 (50%)		
Mediastinal, hyperplasia, lymphoid		1 (50%)			1 (9%)
Mediastinal, infiltration cellular, plasma cell		1 (50%)			
Renal, hyperplasia, lymphoid					1 (9%)
Renal, infiltration cellular, plasma cell			2 (50%)		
Lymph node, mandibular	(47)	(45)	(47)	(47)	(46)
Hemorrhage					1 (2%)
Hyperplasia, lymphoid	4 (9%)		6 (13%)	9 (19%)	6 (13%)
Infiltration cellular, histiocyte				1 (2%)	
Infiltration cellular, plasma cell	1 (2%)	1 (2%)	3 (6%)	4 (9%)	1 (2%)
Infiltration cellular, polymorphonuclear					1 (2%)
Lymph node, mesenteric	(44)	(45)	(47)	(46)	(45)
Angiectasis	6 (14%)	4 (9%)	3 (6%)	5 (11%)	2 (4%)
Erythrophagocytosis				1 (2%)	
Hematopoietic cell proliferation	1 (2%)		1 (2%)		
Hemorrhage	8 (18%)	9 (20%)	1 (2%)	4 (9%)	4 (9%)
Hyperplasia, lymphoid	12 (27%)	14 (31%)	7 (15%)	16 (35%)	11 (24%)
Infiltration cellular, histiocyte		1 (2%)		1 (2%)	
Infiltration cellular, mast cell		1 (2%)		2 (4%)	
Infiltration cellular, plasma cell	1 (2%)				1 (2%)
Infiltration cellular, polymorphonuclear			1 (2%)		
Inflammation, chronic active					1 (2%)
Thrombosis	1 (2%)	1 (2%)			
Sinus, dilatation	4 (9%)	3 (7%)	1 (2%)	3 (7%)	
Spleen	(45)	(47)	(46)	(47)	(45)
Angiectasis	1 (2%)				
Atrophy					1 (2%)
Depletion lymphoid				2 (4%)	
Developmental malformation					1 (2%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Hematopoietic System (continued)					
Spleen					
Hematopoietic cell proliferation	5 (11%)	6 (13%)	9 (20%)	6 (13%)	14 (31%)
Hyperplasia, lymphoid	32 (71%)	32 (68%)	27 (59%)	32 (68%)	21 (47%)
Hyperplasia, stromal					1 (2%)
Necrosis					1 (2%)
Pigmentation			2 (4%)		1 (2%)
Thymus	(43)	(42)	(41)	(42)	(40)
Atrophy	18 (42%)	23 (55%)	21 (51%)	18 (43%)	21 (52%)
Cyst			1 (2%)		
Hyperplasia, lymphoid	1 (2%)		2 (5%)		
Integumentary System					
Skin	(47)	(47)	(47)	(47)	(46)
Fibrosis	2 (4%)		2 (4%)		1 (2%)
Inflammation, suppurative		1 (2%)			
Inflammation, chronic active	1 (2%)	2 (4%)	5 (11%)	2 (4%)	2 (4%)
Mineralization	1 (2%)			1 (2%)	
Ulcer	1 (2%)	3 (6%)	3 (6%)		1 (2%)
Epithelium, hyperplasia		1 (2%)	3 (6%)	2 (4%)	2 (4%)
Sebaceous gland, hyperplasia					1 (2%)
Musculoskeletal System					
Bone, femur	(48)	(48)	(48)	(48)	(48)
Fibro-osseous lesion					1 (2%)
Skeletal muscle	(45)	(46)	(47)	(47)	(44)
Degeneration			1 (2%)		
Nervous System					
Brain, brain stem	(46)	(46)	(47)	(47)	(45)
Mineralization	32 (70%)	27 (59%)	32 (68%)	33 (70%)	27 (60%)
Brain, cerebellum	(46)	(46)	(47)	(47)	(45)
Gliosis		1 (2%)			
Hemorrhage	1 (2%)			1 (2%)	
Necrosis					1 (2%)
Neuron, depletion		1 (2%)			
Brain, cerebrum	(46)	(46)	(47)	(47)	(45)
Hemorrhage	1 (2%)				
Infiltration cellular, mononuclear cell	2 (4%)	1 (2%)	1 (2%)		
Inflammation, suppurative					1 (2%)
Mineralization	28 (61%)	20 (43%)	28 (60%)	27 (57%)	18 (40%)
Hippocampus, gliosis				1 (2%)	
Hippocampus, neuron, depletion				1 (2%)	
Peripheral nerve, sciatic	(46)	(46)	(47)	(47)	(45)
Infiltration cellular, mononuclear cell			2 (4%)		
Axon, degeneration	29 (63%)	26 (57%)	26 (55%)	24 (51%)	24 (53%)
Spinal cord, cervical	(46)	(45)	(47)	(46)	(46)
Axon, degeneration	5 (11%)	4 (9%)	7 (15%)	3 (7)	6 (13%)
Nerve, degeneration					1 (2%)
Neuron, degeneration					1 (2%)
Spinal cord, lumbar	(46)	(45)	(47)	(47)	(46)
Infiltration cellular, mononuclear cell		1 (2%)	2 (4%)		
Axon, degeneration	25 (54%)	24 (53%)	20 (43%)	21 (45%)	19 (41%)
Nerve, degeneration	36 (78%)	34 (76%)	39 (83%)	34 (72%)	35 (76%)
Neuron, degeneration			1 (2%)	1 (2%)	

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Nervous System (continued)					
Spinal cord, thoracic	(46)	(45)	(47)	(47)	(47)
Hemorrhage				1 (2%)	
Infiltration cellular, mononuclear cell				1 (2%)	
Axon, degeneration	38 (83%)	36 (80%)	37 (79%)	36 (77%)	29 (62%)
Neuron, degeneration					1 (2%)
Respiratory System					
Lung	(47)	(46)	(47)	(45)	(48)
Hemorrhage			1 (2%)		
Infiltration cellular, histiocyte	2 (4%)		1 (2%)	1 (2%)	4 (8%)
Inflammation, chronic active	1 (2%)				1 (2%)
Alveolar epithelium, hyperplasia			3 (6%)	4 (9%)	9 (19%)
Nose	(45)	(45)	(47)	(47)	(46)
Hyaline droplet	4 (9%)	6 (13%)	11 (23%)	2 (4%)	2 (4%)
Posterior to upper incisor, dysplasia	1 (2%)				
Special Senses System					
Eye	(44)	(44)	(45)	(44)	(41)
Cataract	3 (7%)	6 (14%)	4 (9%)	6 (14%)	9 (22%)
Phthisis bulbi					1 (2%)
Bilateral, cataract			1 (2%)		
Cornea, inflammation, chronic active			1 (2%)	4 (9%)	2 (5%)
Cornea, ulcer				1 (2%)	
Harderian gland	(46)	(46)	(47)	(47)	47)
Hyperplasia					2 (4%)
Infiltration cellular, lymphocyte	2 (4%)				
Inflammation, chronic active					1 (2%)
Urinary System					
Kidney	(45)	(46)	(47)	(47)	(44)
Autolysis		1 (2%)			
Cyst	1 (2%)				
Hyaline droplet	3 (7%)	2 (4%)			1 (2%)
Hydronephrosis			1 (2%)		
Infarct			1 (2%)		1 (2%)
Infiltration cellular, lymphocyte	22 (49%)	21 (46%)	19 (40%)	19 (40%)	8 (18%)
Inflammation, chronic active					2 (5%)
Metaplasia, osseous		1 (2%)	2 (4%)	1 (2%)	1 (2%)
Mineralization			1 (2%)		
Nephropathy	13 (29%)	8 (17%)	12 (26%)	15 (32%)	16 (36%)
Polyarteritis			1 (2%)		
Capsule, inflammation, chronic active		1 (2%)			
Transitional epithelium, hyperplasia					1 (2%)
Urinary bladder	(46)	(47)	(46)	(45)	(43)
Infiltration cellular, lymphocyte	8 (17%)	2 (4%)	4 (9%)	3 (7%)	1 (2%)
Infiltration cellular, plasma cell			1 (2%)		1 (2%)
Inflammation, chronic active			1 (2%)		2 (5%)
Ulcer					1 (2%)
Lumen, dilatation	3 (7%)	4 (9%)	3 (7%)	3 (7%)	7 (16%)

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

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF ACRYLAMIDE

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Drinking Water Study of Acrylamide[a]

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Disposition Summary					
Animals initially in study	48	48	48	48	48
Early deaths					
Accidental sacrifice			2		
Moribund sacrifice	4	6	8	17	17
Natural deaths	2	4		3	10
Survivors					
Moribund sacrifice	2	1	2	3	5
Natural deaths	1	1			1
Terminal sacrifice	39	36	36	25	15
Animals examined microscopically	48	48	48	48	47
Alimentary System					
Gallbladder	(45)	(43)	(47)	(44)	(37)
Lymphoma malignant				1 (2%)	2 (5%)
Intestine large, cecum	(45)	(44)	(47)	(45)	(37)
Lymphoma malignant		1 (2%)		1 (2%)	2 (5%)
Intestine large, colon	(45)	(44)	(48)	(45)	(37)
Lymphoma malignant					1 (3%)
Intestine large, rectum	(45)	(44)	(47)	(45)	(38)
Histiocytic sarcoma					1 (3%)
Intestine small, duodenum	(45)	(44)	(47)	(45)	(37)
Adenoma		1 (2%)			1 (3%)
Lymphoma malignant					2 (5%)
Intestine small, ileum	(45)	(44)	(47)	(45)	(37)
Lymphoma malignant			1 (2%)	1 (2%)	1 (3%)
Sarcoma stromal, metastatic, uterus				1 (2%)	
Intestine small, jejunum	(45)	(43)	(47)	(44)	(37)
Liver	(47)	(47)	(48)	(46)	(44)
Carcinoma, metastatic, stomach, glandular					1 (2%)
Hemangiosarcoma			1 (2%)		
Hepatocellular adenoma	3 (6%)		2 (4%)	1 (2%)	4 (9%)
Hepatocellular adenoma, multiple					1 (2%)
Hepatocellular carcinoma		2 (4%)	3 (6%)		
Histiocytic sarcoma		4 (9%)		3 (7%)	1 (2%)
Leukemia		1 (2%)	1 (2%)		2 (5%)
Lymphoma malignant	4 (9%)	4 (9%)	1 (2%)	7 (15%)	4 (9%)
Osteosarcoma			1 (2%)		
Mesentery	(0)	(0)	(0)	(1)	(2)
Hemangioma					1 (50%)
Sarcoma					1 (50%)
Pancreas	(46)	(45)	(48)	(45)	(40)
Histiocytic sarcoma				1 (2%)	1 (3%)
Leukemia		1 (2%)	1 (2%)		
Lymphoma malignant	1 (2%)	1 (2%)		3 (7%)	3 (8%)
Sarcoma					1 (3%)
Sarcoma stromal, metastatic, uterus				1 (2%)	
Salivary glands	(47)	(46)	(48)	(45)	(42)
Lymphoma malignant		2 (4%)		1 (2%)	2 (5%)
Adventitia, febrosarcoma, metastatic, skin					1 (2%)
Stomach, forestomach	(46)	(46)	(48)	(45)	(42)
Squamous cell papilloma	4 (9%)		2 (4%)	5 (11%)	6 (14%)
Squamous cell papilloma, multiple					2 (5%)
Stomach, glandular	(45)	(44)	(48)	(45)	(39)
Carcinoma					1 (3%)
Lymphoma malignant				1 (2%)	1 (3%)
Tongue	(1)	(0)	(1)	(1)	(0)
Squamous cell papilloma				1 (100%)	

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice
in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Cardiovascular System					
Blood vessel	(46)	(47)	(48)	(45)	(45)
Lymphoma malignant				1 (2%)	3 (7%)
Heart	(48)	(47)	(48)	(46)	(44)
Leukemia		1 (2%)			1 (2%)
Lymphoma malignant				2 (4%)	3 (7%)
Endocrine System					
Adrenal cortex	(45)	(46)	(48)	(45)	(41)
Histiocytic sarcoma		1 (2%)		1 (2%)	
Leukemia		1 (2%)			
Lymphoma malignant			1 (2%)	2 (4%)	3 (7%)
Adventitia, sarcoma, metastatic, skin				1 (2%)	
Adrenal medulla	(45)	(45)	(48)	(43)	(41)
Lymphoma malignant					1 (2%)
Pheochromocytoma benign				1 (2%)	2 (5%)
Pheochromocytoma malignant			1 (2%)		
Islets, pancreatic	(46)	(46)	(48)	(45)	(40)
Lymphoma malignant				1 (2%)	2 (5%)
Parathyroid gland	(41)	(46)	(45)	(43)	(41)
Pituitary gland	(45)	(45)	(47)	(44)	(42)
Leukemia					1 (2%)
Lymphoma malignant				1 (2%)	3 (7%)
Pars distalis, adenoma	1 (2%)	3 (7%)	3 (6%)		1 (2%)
Pars distalis, carcinoma				1 (2%)	
Thyroid gland	(46)	(46)	(48)	(45)	(41)
Lymphoma malignant					1 (2%)
Follicular cell, adenoma	1 (2%)	1 (2%)			
General Body System					
Tissue NOS	(0)	(0)	(0)	(0)	(3)
Fibrosarcoma, metastatic, skin					1 (33%)
Neurofibrosarcoma, metastatic, skin					1 (33%)
Sarcoma, metastatic, uterus					1 (33%)
Genital System					
Clitoral gland	(44)	(47)	(47)	(45)	(41)
Lymphoma malignant				1 (2%)	1 (2%)
Ovary	(46)	(45)	(48)	(45)	(42)
Cystadenoma	1 (2%)				
Granulosa cell tumor benign		1 (2%)		1 (2%)	5 (12%)
Hemangioma				1 (2%)	
Histiocytic sarcoma		1 (2%)			
Leukemia					1 (2%)
Luteoma			1 (2%)		
Lymphoma malignant				2 (4%)	4 (10%)
Adventitia, sarcoma, metastatic, skin				1 (2%)	
Uterus	(47)	(45)	(48)	(46)	(41)
Adenoma		1 (2%)			
Hemangiosarcoma		1 (2%)			
Histiocytic sarcoma		1 (2%)		3 (7%)	1 (2%)
Leiomyoma	1 (2%)				
Leukemia		1 (2%)			
Lymphoma malignant				1 (2%)	2 (5%)
Polyp stromal					1 (2%)
Sarcoma					2 (5%)
Sarcoma stromal		1 (2%)		1 (2%)	

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Genital System (continued)					
Uterus (continued)					
Squamous cell carcinoma					1 (2%)
Cervix, histiocytic sarcoma		1 (2%)			
Vagina	(0)	(0)	(0)	(1)	(0)
Histiocytic sarcoma				1 (100%)	
Hematopoietic System					
Bone marrow	(45)	(46)	(48)	(45)	(42)
Hemangiosarcoma			1 (2%)		1 (2%)
Histiocytic sarcoma		1 (2%)			
Leukemia		1 (2%)	1 (2%)		1 (2%)
Lymphoma malignant					1 (2%)
Lymph node	(8)	(9)	(4)	(15)	(8)
Lymphoma malignant		1 (11%)			
Axillary, histiocytic sarcoma		1 (11%)			
Axillary, leukemia					1 (13%)
Axillary, liposarcoma, metastatic, skin				1 (7%)	
Axillary, lymphoma malignant	1 (13%)	2 (22%)		2 (13%)	4 (50%)
Brachial, lymphoma malignant				1 (7%)	
Iliac, lymphoma malignant				3 (20%)	1 (13%)
Inguinal, histiocytic sarcoma		1 (11%)			
Inguinal, leukemia					1 (13%)
Inguinal, liposarcoma, metastatic, skin				1 (7%)	
Inguinal, lymphoma malignant	1 (13%)	1 (11%)		2 (13%)	4 (50%)
Lumbar, histiocytic sarcoma		2 (22%)		1 (7%)	1 (13%)
Lumbar, leukemia					1 (13%)
Lumbar, lymphoma malignant	1 (13%)	3 (33%)	2 (50%)	5 (33%)	4 (50%)
Mediastinal, histiocytic sarcoma		1 (11%)			
Mediastinal, lymphoma malignant		1 (11%)	1 (25%)	4 (27%)	1 (13%)
Pancreatic, leukemia					1 (13%)
Pancreatic, lymphoma malignant	2 (25%)	1 (11%)	1 (25%)	2 (13%)	2 (25%)
Popliteal, lymphoma malignant				1 (7%)	
Renal, fibrosarcoma, metastatic, skin				1 (7%)	
Renal, histiocytic sarcoma		2 (22%)			
Renal, liposarcoma, metastatic, skin				1 (7%)	
Renal, lymphoma malignant	1 (13%)	4 (44%)	4 (100%)	6 (40%)	4 (50%)
Lymph node, mandibular	(45)	(47)	(48)	(45)	(41)
Histiocytic sarcoma		2 (4%)			1 (2%)
Leukemia			1 (2%)		1 (2%)
Lymphoma malignant	2 (4%)	7 (15%)	6 (13%)	7 (16%)	5 (12%)
Sarcoma, metastatic, skin					1 (2%)
Lymph node, mesenteric	(44)	(46)	(46)	(44)	(42)
Histiocytic sarcoma		2 (4%)		2 (5%)	1 (2%)
Leukemia					1 (2%)
Lymphoma malignant	3 (7%)	7 (15%)	8 (17%)	6 (14%)	7 (17%)
Spleen	(46)	(46)	(48)	(45)	(44)
Hemangiosarcoma	1 (2%)	1 (2%)	1 (2%)		2 (5%)
Histiocytic sarcoma		2 (4%)		1 (2%)	
Leukemia		1 (2%)	1 (2%)		2 (5%)
Lymphoma malignant	4 (9%)	7 (15%)	7 (15%)	8 (18%)	5 (11%)
Thymus	(40)	(44)	(46)	(45)	(39)
Histiocytic sarcoma		1 (2%)			
Lymphoma malignant	1 (3%)	4 (9%)	5 (11%)	7 (16%)	7 (18%)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Integumentary System					
Mammary gland	(47)	(46)	(48)	(45)	(42)
Adenoacanthoma		1 (2%)	1 (2%)	2 (4%)	4 (10%)
Adenocarcinoma		3 (7%)	5 (10%)	2 (4%)	12 (29%)
Adenocarcinoma, multiple		1 (2%)	1 (2%)		1 (2%)
Adenoma					1 (2%)
Lymphoma malignant				1 (2%)	
Skin	(48)	(46)	(48)	(45)	(43)
Lymphoma malignant				1 (2%)	
Squamous cell carcinoma					2 (5%)
Squamous cell papilloma	1 (2%)			1 (2%)	
Subcutaneous tissue, fibrosarcoma			1 (2%)	4 (9%)	3 (7%)
Subcutaneous tissue, fibrosarcoma, multiple				1 (2%)	
Subcutaneous tissue, fibrous histiocytoma					1 (2%)
Subcutaneous tissue, hemangiosarcoma					1 (2%)
Subcutaneous tissue, liposarcoma				1 (2%)	
Subcutaneous tissue, mast cell tumor malignant, multiple			1 (2%)		
Subcutaneous tissue, myxosarcoma			1 (2%)		
Subcutaneous tissue, neurofibrosarcoma			1 (2%)	1 (2%)	1 (2%)
Subcutaneous tissue, sarcoma				3 (7%)	1 (2%)
Musculoskeletal System					
Bone, femur	(48)	(48)	(48)	(47)	(47)
Osteosarcoma					1 (2%)
Skeletal muscle	(47)	(46)	(48)	(45)	(42)
Lymphoma malignant					1 (2%)
Neurofibrosarcoma, metastatic, skin					1 (2%)
Sarcoma, metastatic, skin				1 (2%)	
Nervous System					
Brain, brain stem	(47)	(47)	(48)	(45)	(42)
Leukemia		1 (2%)			
Lymphoma malignant					1 (2%)
Brain, cerebellum	(47)	(47)	(48)	(45)	(41)
Leukemia		1 (2%)			
Lymphoma malignant					1 (2%)
Brain, cerebrum	(48)	(47)	(48)	(45)	(41)
Leukemia		1 (2%)			
Lymphoma malignant					1 (2%)
Peripheral nerve, sciatic	(46)	(47)	(48)	(45)	(42)
Spinal cord, cervical	(47)	(47)	(48)	(45)	(44)
Leukemia		1 (2%)			
Spinal cord, lumbar	(47)	(47)	(48)	(45)	(45)
Fibrosarcoma, metastatic, skin				1 (2%)	
Leukemia		1 (2%)			
Spinal cord, thoracic	(48)	(47)	(48)	(45)	(44)
Leukemia		1 (2%)			
Respiratory System					
Lung	(47)	(47)	(48)	(45)	(45)
Alveolar/bronchiolar adenoma	1 (2%)	3 (6%)	6 (13%)	10 (22%)	15 (33%)
Alveolar/bronchiolar adenoma, multiple		1 (2%)		1 (2%)	4 (9%)
Alveolar/bronchiolar carcinoma	1 (2%)				1 (2%)
Fibrosarcoma, metastatic, skin			1 (2%)		1 (2%)
Histiocytic sarcoma		2 (4%)		2 (4 %)	
Leukemia		1 (2%)	1 (2%)		2 (4%)
Lymphoma malignant	1 (2%)	3 (6%)		5 (11%)	4 (9%)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Respiratory System (continued)					
Lung (continued)					
Osteosarcoma			1 (2%)		
Sarcoma, metastatic, skin					1 (2%)
Nose	(47)	(46)	(47)	(45)	(43)
Lymphoma malignant			1 (2%)	1 (2%)	1 (2%)
Sarcoma, metastatic, skin					1 (2%)
Special Senses System					
Eye	(45)	(44)	(47)	(45)	(38)
Lymphoma malignant					1 (3%)
Harderian gland	(45)	(44)	(48)	(47)	(43)
Adenoma		8 (18%)	18 (38%)	25 (53%)	16 (37%)
Histiocytic sarcoma		1 (2%)			
Lymphoma malignant					2 (5%)
Bilateral, adenoma			2 (4%)	7 (15%)	15 (35%)
Urinary System					
Kidney	(47)	(46)	(48)	(45)	(40)
Histiocytic sarcoma		1 (2%)		1 (2%)	1 (3%)
Leukemia		1 (2%)			
Lymphoma malignant	1 (2%)	3 (7%)	1 (2%)	5 (11%)	4 (10%)
Osteosarcoma			1 (2%)		
Pelvis, sarcoma, metastatic, skin				1 (2%)	
Ureter	(0)	(0)	(0)	(0)	(1)
Lymphoma malignant					1 (100%)
Urinary bladder	(45)	(45)	(48)	(45)	(38)
Histiocytic sarcoma		1 (2%)			
Lymphoma malignant	1 (2%)			2 (4%)	1 (3%)
Sarcoma stromal, metastatic, uterus				1 (2%)	
Systemic Lesions					
Multiple organs	(48)[b]	(48)[b]	(48)[b]	(48)[b]	(47)[b]
Histiocytic sarcoma		4 (8%)		4 (8%)	1 (2%)
Leukemia		1 (2%)	1 (2%)		2 (4%)
Lymphoma malignant	5 (10%)	7 (15%)	8 (17%)	9 (19%)	8 (17%)
Neoplasm Summary					
Total animals with primary neoplasms[c]	18	30	36	47	46
Total primary neoplasms	20	41	64	83	123
Total animals with benign neoplasms	12	16	28	39	39
Total benign neoplasms	13	19	34	54	75
Total animals with malignant neoplasms	6	20	20	26	34
Total malignant neoplasms	7	22	30	29	48
Total animals with metastatic neoplasms			1	5	5
Total metastatic neoplasms			1	12	10

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

TABLE D2
Statistical Analysis of Neoplasms in Female Mice
in the 2-Year Drinking Water Study of Acrylamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Adrenal Medulla: Benign Pheochromocytoma					
Overall rate[a]	0/45 (0%)	0/45 (0%)	0/48 (0%)	1/43 (2%)	2/41 (5%)
Adjusted rate[b]	0%	0%	0%	2.9%	6.3%
Terminal rate[c]	0/38 (0%)	0/35 (0%)	0/36 (0%)	1/24 (4%)	2/15 (13%)
First incidence (days)[d]	-	-	-	732 (T)	732 (T)
Poly-3 test[e]	P=0.017	-	-	P=0.458	P=0.175
Adrenal Medulla: Malignant Pheochromocytoma					
Overall rate	0/45 (0%)	0/45 (0%)	1/48 (2%)	0/43 (0%)	0/41 (0%)
Adjusted rate	0%	0%	2.3%	0%	0%
Terminal rate	0/38 (0%)	0/35 (0%)	1/36 (3%)	0/24 (0%)	0/15 (0%)
First incidence (days)	-	-	732 (T)	-	-
Poly-3 test	P=0.684N	-	P=0.501	-	-
Adrenal Medulla: Benign or Malignant Pheochromocytoma					
Overall rate	0/45 (0%)	0/45 (0%)	1/48 (2%)	1/43 (2%)	2/41 (5%)
Adjusted rate	0%	0%	2.3%	2.9%	6.3%
Terminal rate	0/38 (0%)	0/35 (0%)	1/36 (3%)	1/24 (4%)	2/15 (13%)
First incidence (days)	-	-	732 (T)	732 (T)	732 (T)
Poly-3 test	P=0.041	-	P=0.501	P=0.458	P=0.175
Harderian Gland: Adenoma					
Overall rate	0/45 (0%)	8/44 (18%)	20/48 (42%)	32/47 (68%)	31/43 (72%)
Adjusted rate	0%	19.0%	44.8%	73.8%	78.8%
Terminal rate	0/39 (0%)	6/35 (17%)	16/36 (44%)	18/25 (72%)	10/15 (67%)
First incidence (days)	-	595	532	474	535
Poly-3 test	P<0.001	P=0.003	P<0.001	P<0.001	P<0.001
Liver: Hepatocellular Adenoma					
Overall rate	3/47 (6%)	0/47 (0%)	2/48 (4%)	1/46 (2%)	5/44 (11%)
Adjusted rate	6.7%	0%	4.6%	2.7%	15.0%
Terminal rate	3/39 (8%)	0/36 (0%)	2/36 (6%)	1/25 (4%)	3/15 (20%)
First incidence (days)	732 (T)	-	732 (T)	732 (T)	718
Poly-3 test	P=0.040	P=0.122N	P=0.518N	P=0.378N	P=0.209
Liver: Hepatocellular Carcinoma					
Overall rate	0/47 (0%)	2/47 (4%)	3/48 (6%)	0/46 (0%)	0/44 (0%)
Adjusted rate	0.0%	4.6%	7.0%	0.0%	0.0%
Terminal rate	0/39 (0%)	2/36 (6%)	3/36 (8%)	0/25 (0%)	0/15 (0%)
First incidence (days)	-	725 (T)	732 (T)	-	-
Poly-3 test	P=0.305N	P=0.232	P=0.112	-	-
Liver: Hepatocellular Adenoma or Carcinoma					
Overall rate	3/47 (6%)	2/47 (4%)	5/48 (10%)	1/46 (2%)	5/44 (11%)
Adjusted rate	6.7%	4.6%	11.6%	2.7%	15.0%
Terminal rate	3/39 (8%)	2/36 (6%)	5/36 (14%)	1/25 (4%)	3/15 (20%)
First incidence (days)	732 (T)	725 (T)	732 (T)	732 (T)	718
Poly-3 test	P=0.158	P=0.509N	P=0.335	P=0.378N	P=0.209
Lung: Alveolar/bronchiolar Adenoma					
Overall rate	1/47 (2%)	4/47 (9%)	6/48 (13%)	11/45 (24%)	19/45 (42%)
Adjusted rate	2.2%	9.0%	13.8%	29.5%	52.7%
Terminal rate	1/39 (3%)	1/36 (3%)	4/36 (11%)	10/25 (40%)	11/15 (73%)
First incidence (days)	732 (T)	595	645	483	537
Poly-3 test	P<0.001	P=0.177	P=0.051	P<0.001	P<0.001

TABLE D2
Statistical Analysis of Neoplasms in Female Mice
in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Lung: Alveolar/bronchiolar Carcinoma					
Overall rate	1/47 (2%)	0/47 (0%)	0/48 (0%)	0/45 (0%)	1/45 (2%)
Adjusted rate	2.2%	0%	0%	0%	2.9%
Terminal rate	1/39 (3%)	0/36 (0%)	0/36 (0%)	0/25 (0%)	1/15 (7%)
First incidence (days)	732 (T)	-	-	-	732 (T)
Poly-3 test	P=0.461	P=0.504N	P=508N	P=540N	P=697
Lung: Alveolar/bronchiolar Adenoma or Carcinoma					
Overall rate	2/47 (4%)	4/47 (9%)	6/48 (13%)	11/45 (24%)	20/45 (44%)
Adjusted rate	4.5%	9.0%	13.8%	29.5%	55.4%
Terminal rate	2/39 (5%)	1/36 (3%)	4/36 (11%)	10/25 (40%)	12/15 (80%)
First incidence (days)	732 (T)	595	645	483	537
Poly-3 test	P<0.001	P=0.335	P=0.123	P=0.002	P<0.001
Mammary Gland: Adenoma					
Overall rate[a]	0/47 (0%)	0/46 (0%)	0/48 (0%)	0/45 (0%)	1/42 (2%)
Adjusted rate[b]	0%	0%	0%	0%	2.9%
Terminal rate[c]	0/39 (0%)	0/36 (0%)	0/36 (0%)	0/25 (0%)	0/15 (0%)
First incidence (days)[d]	-	-	-	-	519
Poly-3 test[e]	P=0.118	-	-	-	P=0.448
Mammary Gland: Adenoacanthoma/Adenocarcinoma					
Overall rate	0/47 (0%)	4/46 (9%)	7/48 (15%)	4/45 (9%)	17/42 (41%)
Adjusted rate	0%	9.1%	16.0%	10.5%	45.4%
Terminal rate	0/39 (0%)	1/36 (3%)	4/36 (11%)	0/25 (0%)	5/15 (33%)
First incidence (days)	-	625	6645	596	535
Poly-3 test	P<0.001	P=0.059	P=0.007	P=0.042	P<0.001
Mammary Gland: Adenoma or Adenoacanthoma/Adenocarcinoma					
Overall rate	0/47 (0%)	4/46 (9%)	7/48 (15%)	4/45 (9%)	18/42 (43%)
Adjusted rate	0%	9.1%	16.0%	10.5%	47.3%
Terminal rate	0/39 (0%)	1/36 (3%)	4/36 (11%)	0/25 (0%)	5/15 (33%)
First incidence (days)	-	625	645	596	519
Poly-3 test	P<0.001	P=0.059	P=0.007	P=0.042	P<0.001
Ovary: Benign Granulosa Cell Tumor					
Overall rate	0/46 (0%)	1/45 (2%)	0/48 (0%)	1/45 (2%)	5/42 (12%)
Adjusted rate	0%	2.4%	0%	2.7%	15.4%
Terminal rate	0/39 (0%)	1/36 (3%)	0/36 (0%)	0/25 (0%)	3/15 (20%)
First incidence (days)	-	732 (T)	-	642	673
Poly-3 test	P<0.001	P=0.491	-	P=0.464	P=0.012
Pituitary Gland (Pars Distalis): Adenoma					
Overall rate	1/45 (2%)	3/45 (7%)	3/47 (6%)	0/44 (0%)	1/42 (2%)
Adjusted rate	2.3%	7.1%	7.1%	0.0%	3.1%
Terminal rate	1/38 (3%)	3/35 (9%)	3/35 (9%)	0/25 (0%)	1/15 (7%)
First incidence (days)	732 (T)	725 (T)	732 (T)	-	732 (T)
Poly-3 test	P=0.363N	P=0.297	P=0.296	P=0.536N	P=0.694
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma					
Overall rate	1/45 (2%)	3/45 (7%)	3/47 (6%)	1/44 (2%)	1/42 (2%)
Adjusted rate	2.3%	7.1%	7.1%	2.7%	3.1%
Terminal rate	1/38 (3%)	3/35 (9%)	3/35 (9%)	0/25 (0%)	1/15 (7%)
First incidence (days)	732 (T)	725 (T)	732 (T)	602	732 (T)
Poly-3 test	P=0.438N	P=0.297	P=0.296	P=0.722	P=0.694

TABLE D2
Statistical Analysis of Neoplasms in Female Mice
in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Skin: Fibrosarcoma					
Overall rate	0/48 (0%)	0/46 (0%)	1/48 (2%)	5/45 (11%)	3/43 (7%)
Adjusted rate	0.0%	0.0%	2.3%	13.5%	8.7%
Terminal rate	0/39 (0%)	0/36 (0%)	0/36 (0%)	3/25 (12%)	0/15 (0%)
First incidence (days)	-	-	730	670	635
Poly-3 test	P=0.006	-	P=0.490	P=0.016	P=0.075
Skin: Fibrosarcoma, Sarcoma, Myxosarcoma, or Fibrous Histiocytoma					
Overall rate	0/48 (0%)	0/46 (0%)	2/48 (4%)	8/45 (18%)	5/43 (12%)
Adjusted rate	0.0%	0.0%	4.6%	21.2%	14.3%
Terminal rate	0/39 (0%)	0/36 (0%)	1/36 (3%)	4/25 (16%)	0/15 (0%)
First incidence (days)	-	-	730	534	621
Poly-3 test	P<.001	-	P=0.226	P=0.001	P=0.014
Skin: Fibrosarcoma, Hemangiosarcoma, Liposarcoma, Myxosarcoma, Neurofibrosarcoma, or Sarcoma					
Overall rate	0/48 (0%)	0/46 (0%)	3/48 (6%)	10/45 (22%)	6/43 (14%)
Adjusted rate	0%	0%	6.9%	26.2%	17.1%
Terminal rate	0/39 (0%)	0/36 (0%)	1/36 (3%)	5/25 (20%)	0/15 (0%)
First incidence (days)	-	-	708	534	614
Poly-3 test	P<0.001	-	P=0.110	P<0.001	P=0.005
Skin: Squamous Cell Carcinoma					
Overall rate	0/48 (0%)	0/46 (0%)	0/48 (0%)	0/45 (0%)	2/43 (5%)
Adjusted rate	0%	0%	0%	0%	5.9%
Terminal rate	0/39 (0%)	0/36 (0%)	0/36 (0%)	0/25 (0%)	1/15 (7%)
First incidence (days)	-	-		-	680
Poly-3 test	P=0.015	-	-	-	P=0.177
Skin: Squamous Cell Papilloma					
Overall rate	1/48 (2%)	0/46 (0%)	0/48 (0%)	1/45 (2%)	0/43 (0%)
Adjusted rate	2.2%	0.0%	0.0%	2.7%	0.0%
Terminal rate	1/39 (3%)	0/36 (0%)	0/36 (0%)	1/25 (4%)	0/15 (0%)
First incidence (days)	732 (T)	-	-	732 (T)	-
Poly-3 test	P=0.545N	P=0.511N	P=0.510N	P=0.711	P=0.558N
Skin: Squamous Cell Carcinoma or Papilloma					
Overall rate	1/48 (2%)	0/46 (0%)	0/48 (0%)	1/45 (2%)	2/43 (5%)
Adjusted rate	2.2%	0.0%	0.0%	2.7%	5.9%
Terminal rate	1/39 (3%)	0/36 (0%)	0/36 (0%)	1/25 (4%)	1/15 (7%)
First incidence (days)	732 (T)	-	-	732 (T)	680
Poly-3 test	P=0.101	P=0.511N	P=0.510N	P=0.711	P=0.400
Skin: Sarcoma					
Overall rate	0/48 (0%)	0/46 (0%)	0/48 (0%)	3/45 (7%)	1/43 (2%)
Adjusted rate	0.0%	0.0%	0.0%	8.0%	2.9%
Terminal rate	0/39 (0%)	0/36 (0%)	0/36 (0%)	1/25 (4%)	0/15 (0%)
First incidence (days)	-	-	-	534	677
Poly-3 test	P=0.080	-	-	P=0.087	P=0.443
Skin: All Morphologies					
Overall rate	1/48 (2%)	0/46 (0%)	4/48 (8%)	11/45 (24%)	9/43 (21%)
Adjusted rate	2.2%	0.0%	9.2%	28.8%	25.3%
Terminal rate	1/39 (3%)	0/36 (0%)	1/36 (3%)	6/25 (24%)	1/15 (7%)
First incidence (days)	732 (T)	-	664	534	614
Poly-3 test	P<.001	P=0.511N	P=0.166	P=<.001	P=0.002

TABLE D2
Statistical Analysis of Neoplasms in Female Mice
in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Stomach (Forestomach): Squamous Cell Papilloma					
Overall rate	4/46 (9%)	0/46 (0%)	2/48 (4%)	5/45 (11%)	8/42 (19%)
Adjusted rate	9.1%	0%	4.6%	13.3%	24.0%
Terminal rate	4/39 (10%)	0/36 (0%)	1/36 (3%)	3/25 (12%)	4/15 (27%)
First incidence (days)	732 (T)	-	692	483	583
Poly-3 test	P=0.001	P=0.063N	P=0.344N	P=0.402	P=0.070
Uterus: Sarcoma					
Overall rate	0/47 (0%)	0/45 (0%)	0/48 (0%)	0/46 (0%)	2/41 (5%)
Adjusted rate	0%	0%	0%	0%	6.1%
Terminal rate	0/39 (0%)	0/36 (0%)	0/36 (0%)	0/25 (0%)	0/15 (0%)
First incidence (days)	-	-	-	-	628
Poly-3 test	P=0.013	-	-	-	P=0.172
All Organs: Fibrous Histiocytoma					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/47 (2%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	2.8%
Terminal rate	0/39 (0%)	0/36 (0%)	0/36 (0%)	0/25 (0%)	0/15 (0%)
First incidence (days)	-	-	-	-	621
Poly-3 test	P=0.124	-	-	-	P=0.453
All Organs: Hemangioma					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)	1/47 (2%)
Adjusted rate	0.0%	0.0%	0.0%	2.6%	2.8%
Terminal rate	0/39 (0%)	0/36 (0%)	0/36 (0%)	0/25 (0%)	0/15 (0%)
First incidence (days)	-	-	-	596	726
Poly-3 test	P=0.119	-	-	P=0.464	P=0.451
All Organs: Hemangiosarcoma					
Overall rate	1/48 (2%)	2/48 (4%)	1/48 (2%)	0/48 (0%)	2/47 (4%)
Adjusted rate	2.2%	4.6%	2.3%	0.0%	5.6%
Terminal rate	0/39 (0%)	2/36 (6%)	1/36 (3%)	0/25 (0%)	0/15 (0%)
First incidence (days)	586	732 (T)	732 (T)	-	614
Poly-3 test	P=0.418	P=0.485	P=0.747	P=0.541N	P=0.416
All Organs: Hemangioma or Hemangiosarcoma					
Overall rate	1/48 (2%)	2/48 (4%)	1/48 (2%)	1/48 (2%)	3/47 (6%)
Adjusted rate	2.2%	4.6%	2.3%	2.6%	8.4%
Terminal rate	0/39 (0%)	2/36 (6%)	1/36 (3%)	0/25 (0%)	0/15 (0%)
First incidence (days)	586	732 (T)	732 (T)	596	614
Poly-3 test	P=0.157	P=0.485	P=0.747	P=0.716	P=0.225
All Organs: Histiocytic Sarcoma					
Overall rate	0/48 (0%)	4/48 (8%)	0/48 (0%)	4/48 (8%)	1/47 (2%)
Adjusted rate	0.0%	8.9%	0.0%	10.3%	2.8%
Terminal rate	0/39 (0%)	0/36 (0%)	0/36 (0%)	1/25 (4%)	0/15 (0%)
First incidence (days)	-	595	-	474	568
Poly-3 test	P=0.407	P=0.059	-	P=0.043	P=0.454
All Organs: Leukemia					
Overall rate	0/48 (0%)	1/48 (2%)	1/48 (2%)	0/48 (0%)	2/47 (4%)
Adjusted rate	0.0%	2.3%	2.3%	0.0%	5.5%
Terminal rate	0/39 (0%)	0/36 (0%)	0/36 (0%)	0/25 (0%)	0/15 (0%)
First incidence (days)	-	679	645	-	604
Poly-3 test	P=0.141	P=0.495	P=0.491	-	P=0.190

TABLE D2
Statistical Analysis of Neoplasms in Female Mice
in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
All Organs: Malignant Lymphoma					
Overall rate	5/48 (10%)	7/48 (15%)	8/48 (17%)	9/48 (19%)	8/47 (17%)
Adjusted rate	11.0%	15.8%	18.2%	22.5%	20.5%
Terminal rate	4/39 (10%)	5/36 (14%)	5/36 (14%)	4/25 (16%)	1/15 (7%)
First incidence (days)	716	607	614	214	289
Poly-3 test	P=0.146	P=0.363	P=0.255	P=0.128	P=0.184
All Organs: Osteosarcoma or Osteoma					
Overall rate	0/48 (0%)	0/48 (0%)	1/48 (2%)	0/48 (0%)	1/47 (2%)
Adjusted rate	0.0%	0.0%	2.3%	0.0%	2.8%
Terminal rate	0/39 (0%)	0/36 (0%)	1/36 (3%)	0/25 (0%)	0/15 (0%)
First incidence (days)	-	-	732 (T)	-	681
Poly-3 test	P=0.251	-	P=0.490	-	P=0.452
All Organs: Benign Tumors					
Overall rate[a]	12/48 (25%)	16/48 (33%)	28/48 (58%)	39/48 (81%)	39/47 (83%)
Adjusted rate[b]	26.5%	35.5%	62.5%	89.5%	91.9%
Terminal rate[c]	11/39 (28%)	12/36 (33%)	23/36 (64%)	24/25 (96%)	15/15 (100%)
First incidence (days)[d]	730	595	532	474	519
Poly-3 test[e]	P<0.001	P=0.240	P<0.001	P<0.001	P<0.001
All Organs: Malignant Tumors					
Overall rate	6/48 (13%)	20/48 (42%)	20/48 (42%)	26/48 (54%)	34/47 (72%)
Adjusted rate	13.1%	42.9%	44.8%	57.6%	76.3%
Terminal rate	4/39 (10%)	10/36 (28%)	13/36 (36%)	8/25 (32%)	8/15 (53%)
First incidence (days)	586	595	614	214	289
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
All Organs: Benign or Malignant Tumors					
Overall rate	18/48 (38%)	30/48 (63%)	36/48 (75%)	47/48 (98%)	46/47 (98%)
Adjusted rate	39.2%	63.8%	78.6%	97.9%	98.9%
Terminal rate	15/39 (39%)	19/36 (53%)	27/36 (75%)	24/25 (96%)	15/15 (100%)
First incidence (days)	586	595	532	214	289
Poly-3 test	P<0.001	P=0.013	P<0.001	P<0.001	P<0.001

[a] Number of animals with neoplasm per number of animals examined microscopically.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[c] Observed incidence at the terminal sacrifice.
[d] T indicates terminal sacrifice.
[e] Beneath the 0 mM acrylamide are the p values associated with the trend test. Beneath the treated groups incidences are the p values corresponding to pairwise comparisons between the 0 mM acrylamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice. An N indicates a negative trend or decreased tumor incidence.

TABLE D3a
Historical Incidence of Alveolar/Bronchiolar Adenoma in NCTR Control Female B6C3F1 Mice

Study (Report Date)	Route of Administration	Incidence in Controls
Chloral Hydrate (July 2001)	Gavage	8/143 (5.6%)
Doxylamine (April 1991)	Diet	3/48 (6.3%)
Fumonisin B_1 (March 1999)	Diet	2/47 (4.3%)
Leucomalachite Green (June 2001)	Diet	3/47 (6.4%)
Malachite Green (June 2001)	Diet	2/48 (4.2%)
Pyrilamine (July 1991)	Diet	1/48 (2.1%)
Sulfamethazine (February 1988)	Diet	5/182 (2.7%)
Tripolidine (June 1991)	Diet	3/47 (6.4%)
Urethane and Ethanol (May 2003)	Drinking Water	4/48 (8.3%)
Total (%)		31/658 (4.7%)
Range		2.1%-8.3%

TABLE D3b
Historical Incidence of Mammary Gland Neoplasms in NCTR Control Female B6C3F1 Mice

		Incidence in Controls	
Study (Report Date)	**Route of Administration**	**Adenocarcinoma**	**Adenoacanthoma**
Chloral Hydrate (July 2001)	Gavage	1/133 (0.8%)	0/133 (0.0%)
Doxylamine (April 1991)	Diet	4/47 (8.5%)	0/47 (0.0%)
Fumonisin B_1 (March 1999)	Diet	1/46 (2.2%)	0/46 (0.0%)
Leucomalachite Green (June 2001)	Diet	1/46 (2.2%)	2/46 (4.3%)
Malachite Green (June 2001)	Diet	2/46 (4.3%)	0/46 (0.0%)
Pyrilamine (July 1991)	Diet	0/47 (0.0%)	0/47 (0.0%)
Sulfamethazine (February 1988)	Diet	-[a]	-
Tripolidine (June 1991)	Diet	0/44 (0.0%)	0/44 (0.0%)
Urethane and Ethanol (May 2003)	Drinking Water	4/47 (8.5%)	0/47 (0.0%)
Total (%)		13/456 (2.9%)	2/456 (0.4%)
Range		0.0%-8.5%	0.0%-4.3%

[a] Not reported.

TABLE D3c
Historical Incidence of Adenoma of the Harderian Gland in NCTR Control Female B6C3F1 Mice

Study (Report Date)	Route of Administration	Incidence in Controls
Chloral Hydrate (July 2001)	Gavage	4/140 (2.9%)
Doxylamine (April 1991)	Diet	1/48 (2.1%)
Fumonisin B_1 (March 1999)	Diet	4/46 (8.7%)
Leucomalachite Green (June 2001)	Diet	2/47 (4.3%)
Malachite Green (June 2001)	Diet	3/48 (6.3%)
Pyrilamine (July 1991)	Diet	-[a]
Sulfamethazine (February 1988)	Diet	13/182 (7.1%)
Tripolidine (June 1991)	Diet	2/47 (4.3%)
Urethane and Ethanol (May 2003)	Drinking Water	3/48 (6.3%)
Total (%)		32/606 (5.3%)
Range		2.1%-8.7%

[a] Not reported.

TABLE D3d
Historical Incidence of Hepatocellular Adenoma in NCTR Control Female B6C3F1 Mice

Study (Report Date)	Route of Administration	Incidence in Controls
Chloral Hydrate (July 2001)	Gavage	5/144 (3.5%)
Doxylamine (April 1991)	Diet	0/46 (0.0%)
Fumonisin B_1 (March 1999)	Diet	5/47 (10.6%)
Leucomalachite Green (June 2001)	Diet	3/47 (6.4%)
Malachite Green (June 2001)	Diet	3/48 (6.3%)
Pyrilamine (July 1991)	Diet	1/47 (2.1%)
Sulfamethazine (February 1988)	Diet	8/184 (4.3%)
Tripolidine (June 1991)	Diet	2/47 (4.3%)
Urethane and Ethanol (May 2003)	Drinking Water	5/48 (10.4%)
Total (%)		32/658 (4.9%)
Range		0.0%-10.6%

TABLE D3e
Historical Incidence of Benign Granulosa Cell Tumors of the Ovaries in NCTR Control Female B6C3F1 Mice

Study (Report Date)	Route of Administration	Incidence in Controls
Chloral Hydrate (July 2001)	Gavage	1/141 (0.7%)
Doxylamine (April 1991)	Diet	0/47 (0.0%)
Fumonisin B_1 (March 1999)	Diet	0/46 (0.0%)
Leucomalachite Green (June 2001)	Diet	0/46 (0.0%)
Malachite Green (June 2001)	Diet	0/48 (0.0%)
Pyrilamine (July 1991)	Diet	0/48 (0.0%)
Sulfamethazine (February 1988)	Diet	0/177 (0.0%)
Tripolidine (June 1991)	Diet	0/45 (0.0%)
Urethane and Ethanol (May 2003)	Drinking Water	0/48 (0.0%)
Total (%)		1/646 (0.2%)
Range		0.0%-0.7%

TABLE D3f
Historical Incidence of Squamous Cell Papilloma or Carcinoma (Combined) of the Forestomach in NCTR Control Female B6C3F1 Mice

Study (Report Date)	Route of Administration	Incidence in Controls
Chloral Hydrate (July 2001)	Gavage	1/139 (0.7%)
Doxylamine (April 1991)	Diet	0/47 (0.0%)
Fumonisin B_1 (March 1999)	Diet	0/46 (0.0%)
Leucomalachite Green (June 2001)	Diet	0/46 (0.0%)
Malachite Green (June 2001)	Diet	2/47 (4.3%)
Pyrilamine (July 1991)	Diet	1/48 (2.1%)
Sulfamethazine (February 1988)	Diet	1/178 (0.6%)
Tripolidine (June 1991)	Diet	1/46 (2.2%)
Urethane and Ethanol (May 2003)	Drinking Water	2/48 (4.2%)
Total (%)		8/645 (1.2%)
Range		0.0%-4.3%

TABLE D3g
Historical Incidence of Malignant Mescenchymal Skin Tumors in NCTR Control Female B6C3F1 Mice

		Incidence in Controls	
Study (Report Date)	**Route of Administration**	**Fibrosarcoma, Fibrous Histiocytoma, Myxosarcoma, or Sarcoma**	**Neurofibrosarcoma**
Chloral Hydrate (July 2001)	Gavage	1/139 (0.7%)	0/139 (0.0%)
Doxylamine (April 1991)	Diet	1/48 (2.1%)	0/48 (0.0%)
Fumonisin B_1 (March 1999)	Diet	1/47 (2.1%)	0/47 (0.0%)
Leucomalachite Green (June 2001)	Diet	0/46 (0.0%)	0/46 (0.0%)
Malachite Green (June 2001)	Diet	0/48 (0.0%)	0/48 (0.0%)
Pyrilamine (July 1991)	Diet	1/48 (2.1%)	0/48 (0.0%)
Sulfamethazine (February 1988)	Diet	0/181 (0.0%)	0/181 (0.0%)
Tripolidine (June 1991)	Diet	0/46 (0.0%)	0/46 (0.0%)
Urethane and Ethanol (May 2003)	Drinking Water	4/48 (8.3%)	0/48 (0.0%)
Total (%)		4/650 (0.6%)	0/650 (0.0%)
Range		0.0% - 8.3%	0.0%

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Acrylamide[a]

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Disposition Summary					
Animals initially in study	48	48	48	48	48
Early deaths					
Accidentally killed			2		
Moribund sacrifice	4	6	8	17	17
Natural deaths	2	4		3	10
Survivors					
Moribund sacrifice	2	1	2	3	5
Natural deaths	1	1			1
Terminal sacrifice	39	36	36	25	15
Animals examined microscopically	48	48	48	48	47
Alimentary System					
Gallbladder	(45)	(43)	(47)	(44)	(37)
Lumen, dilatation	2 (4%)				
Intestine large, cecum	(45)	(44)	(47)	(45)	(37)
Hyperplasia, lymphoid	2 (4%)	1 (2%)			1 (3%)
Epithelium, hyperplasia					1 (3%)
Intestine large, colon	(45)	(44)	(48)	(45)	(37)
Intestine large, rectum	(45)	(44)	(47)	(45)	(38)
Intestine small, duodenum	(45)	(44)	(47)	(45)	(37)
Hyperplasia, lymphoid					1 (3%)
Intestine small, ileum	(45)	(44)	(47)	(45)	(37)
Hyperplasia, lymphoid	1 (2%)				
Intestine small, jejunum	(45)	(43)	(47)	(44)	(37)
Hyperplasia, lymphoid	1 (2%)	1 (2%)			
Liver	(47)	(47)	(48)	(46)	(44)
Angiectasis		1 (2%)		1 (2%)	2 (5%)
Autolysis				1 (2%)	2 (5%)
Basophilic focus				1 (2%)	2 (5%)
Eosinophilic focus			3 (6%)		1 (2%)
Hematopoietic cell proliferation	3 (6%)	1 (2%)	2 (4%)	5 (11%)	1 (2%)
Hemorrhage		1 (2%)			
Infiltration cellular, lymphocyte	7 (15%)	12 (26%)	10 (21%)	9 (20%)	1 (2%)
Infiltration cellular, mast cell			1 (2%)		
Inflammation, suppurative	1 (2%)				
Inflammation, chronic active		2 (4%)		2 (4%)	5 (11%)
Mineralization					1 (2%)
Necrosis	3 (6%)	2 (4%)	2 (4%)	1 (2%)	3 (7%)
Polyarteritis	1 (2%)				
Tension lipidosis			1 (2%)		1 (2%)
Vacuolization cytoplasmic	5 (11%)		2 (4%)	4 (9%)	1 (2%)
Oval cell, hyperplasia		1 (2%)			
Mesentery	(0)	(0)	(0)	(1)	(2)
Fat, necrosis				1 (100%)	
Pancreas	(46)	(45)	(48)	(45)	(40)
Cyst	1 (2%)				
Infiltration cellular, lymphocyte	6 (13%)	7 (16%)	7 (15%)	5 (11%)	3 (8%)
Inflammation, chronic active	1 (2%)				
Polyarteritis	1 (2%)				
Acinus, degeneration	2 (4%)		2 (4%)	1 (2%)	
Duct, dilatation	1 (2%)				
Salivary glands	(47)	(46)	(48)	(45)	(42)
Hyperplasia			1 (2%)		
Infiltration cellular, lymphocyte	23 (49%)	28 (61%)	25 (52%)	24 (53%)	17 (40%)
Acinus, degeneration			1 (2%)		

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Alimentary System (continued)					
Stomach, forestomach	(46)	(46)	(48)	(45)	(42)
Autolysis					1 (2%)
Diverticulum		1 (2%)			
Keratin cyst	2 (4%)				
Ulcer	2 (4%)	3 (7%)		1 (2%)	
Epithelium, hyperplasia	5 (11%)	9 (20%)	4 (8%)	4 (9%)	11 (26%)
Serosa, inflammation, chronic active	1 (2%)				
Stomach, glandular	(45)	(44)	(48)	(45)	(39)
Autolysis					1 (3%)
Cyst				1 (2%)	
Epithelium, hyperplasia				2 (4%)	1 (3%)
Tongue	(1)	(0)	(1)	(1)	(0)
Inflammation, chronic active			1 (100%)		
Ulcer			1 (100%)		
Cardiovascular System					
Blood vessel	(46)	(47)	(48)	(45)	(45)
Heart	(48)	(47)	(48)	(46)	(44)
Cardiomyopathy					1 (2%)
Inflammation, suppurative	1 (2%)				
Polyarteritis	1 (2%)				
Thrombosis					1 (2%)
Endocrine System					
Adrenal cortex	(45)	(46)	(48)	(45)	(41)
Accessory adrenal cortical nodule	1 (2%)				2 (5%)
Angiectasis		1 (2%)			
Hypertrophy	1 (2%)		1 (2%)	1 (2%)	
Infiltration cellular, polymorphonuclear		1 (2%)			
Vacuolization cytoplasmic	1 (2%)	1 (2%)			
Subcapsular, hyperplasia	45 (100%)	45 (98%)	48 (100%)	45 (100%)	38 (93%)
Adrenal medulla	(45)	(45)	(48)	(43)	(41)
Mineralization	1 (2%)				
Islets, pancreatic	(46)	(46)	(48)	(45)	(40)
Hyperplasia		1 (2%)	1 (2%)	2 (4%)	2 (5%)
Parathyroid gland	(41)	(46)	(45)	(43)	(41)
Cyst	1 (2%)		2 (4%)	1 (2%)	
Pituitary gland	(45)	(45)	(47)	(44)	(42)
Angiectasis		1 (2%)			
Compression	1 (2%)				
Pars distalis, cyst		1 (2%)			
Pars distalis, hyperplasia		2 (4%)	2 (4%)	4 (9%)	2 (5%)
Thyroid gland	(46)	(46)	(48)	(45)	(41)
Cyst	1 (2%)		1 (2%)	2 (4%)	1 (2%)
Ectopic thymus	1 (2%)	2 (4%)			
Infiltration cellular, lymphocyte	2 (4%)	1 (2%)	1 (2%)	2 (4%)	2 (5%)
Polyarteritis	1 (2%)				
Follicle, degeneration	2 (4%)	2 (4%)	7 (15%)	6 (13%)	2 (5%)
Follicular cell, hyperplasia		1 (2%)			
General Body System					
Tissue NOS	(0)	(0)	(0)	(0)	(3)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Genital System					
Clitoral Gland	(44)	(47)	(47)	(45)	(41)
Atrophy	1 (2%)			1 (2%)	
Degeneration	43 (98%)	47 (100%)	45 (96%)	43 (96%)	38 (93%)
Infiltration cellular, lymphocyte				1 (2%)	
Inflammation, suppurative					1 (2%)
Ovary	(46)	(45)	(48)	(45)	(42)
Angiectasis		2 (4%)	1 (2%)	1 (2%)	4 (10%)
Atrophy	45 (98%)	43 (96%)	45 (94%)	38 (84%)	30 (71%)
Cyst	4 (9%)	14 (31%)	10 (21%)	17 (38%)	17 (40%)
Degeneration				1 (2%)	
Hemorrhage		1 (2%)		1 (2%)	4 (10%)
Thrombosis		1 (2%)	1 (2%)	2 (4%)	3 (7%)
Bilateral, cyst	4 (9%)	4 (9%)	2 (4%)	3 (7%)	1 (2%)
Fat, necrosis				1 (2%)	
Granulosa cell, hyperplasia					1 (2%)
Uterus	(47)	(45)	(48)	(46)	(41)
Angiectasis	1 (2%)		1 (2%)		1 (2%)
Autolysis				1 (2%)	
Edema					2 (5%)
Hemorrhage		1 (2%)	1 (2%)		2 (5%)
Hydrometra	4 (9%)	2 (4%)	4 (8%)	1 (2%)	1 (2%)
Hyperplasia, stromal					1 (2%)
Infiltration cellular, lymphocyte	1 (2%)				
Inflammation, suppurative	1 (2%)				
Necrosis	1 (2%)				
Thrombus					2 (5%)
Endometrium, hyperplasia, cystic	43 (91%)	42 (93%)	41 (85%)	38 (83%)	30 (73%)
Vagina	(0)	(0)	(0)	(1)	(0)
Hematopoietic System					
Bone marrow	(45)	(46)	(48)	(45)	(42)
Hyperplasia	2 (4%)	4 (9%)	1 (2%)	5 (11%)	6 (14%)
Lymph node	(8)	(9)	(4)	(15)	(8)
Axillary, hyperplasia, lymphoid		1 (11%)			
Inguinal, hyperplasia, lymphoid		1 (11%)		1 (7%)	
Lumbar, hyperplasia, lymphoid	5 (63%)	2 (22%)		1 (7%)	1 (13%)
Lumbar, infiltration cellular, plasma cell	2 (25)				
Lumbar, infiltration cellular, polymorphonuclear				1 (7%)	
Mediastinal, hyperplasia, lymphoid				1 (7%)	
Pancreatic, hemorrhage		1 (11%)			
Pancreatic, hyperplasia, lymphoid		1 (11%)			1 (13%)
Renal, hyperplasia, lymphoid		3 (33%)		1 (7%)	1 (13%)
Renal, infiltration cellular, plasma cell	1 (13%)				
Renal, infiltration cellular, polymorphonuclear				1 (7%)	
Thoracic, infiltration cellular, plasma cell				1 (7%)	
Lymph node, mandibular	(45)	(47)	(48)	(45)	(41)
Hematopoietic cell proliferation		1 (2%)			
Hyperplasia, lymphoid	5 (11%)	10 (21%)	15 (31%)	13 (29%)	6 (15%)
Infiltration cellular, plasma cell		1 (2%)	3 (6%)	3 (7%)	1 (2%)
Lymph node, mesenteric	(44)	(46)	(46)	(44)	(42)
Angiectasis			1 (2%)		1 (2%)
Hematopoietic cell proliferation			1 (2%)		
Hemorrhage			1 (2%)		1 (2%)
Hyperplasia, lymphoid	12 (27%)	11 (24%)	12 (26%)	8 (18%)	5 (12%)
Infiltration cellular, histiocyte				1 (2%)	
Infiltration cellular, plasma cell			1 (2%)		

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Hematopoietic System (continued)					
Lymph node, mesenteric					
Inflammation, chronic active			1 (2%)		
Polyarteritis	1 (2%)				
Sinus, dilatation					1 (2%)
Spleen	(46)	(46)	(48)	(45)	(44)
Angiectasis		1 (2%)			
Autolysis					1 (2%)
Hematopoietic cell proliferation	5 (11%)	10 (22%)	6 (13%)	14 (31%)	18 (41%)
Hyperplasia, lymphoid	38 (83%)	33 (72%)	34 (71%)	24 (53%)	20 (45%)
Infiltration cellular, mast cell			1 (2%)		
Pigmentation	1 (2%)				
Thymus	(40)	(44)	(46)	(45)	(39)
Angiectasis				1 (2%)	
Atrophy	23 (58%)	16 (36%)	15 (33%)	17 (38%)	16 (41%)
Cyst			1 (2%)		
Hyperplasia, lymphoid	5 (13%)	4 (9%)	6 (13%)	2 (4%)	1 (3%)
Epithelium, hyperplasia	1 (3%)				
Integumentary System					
Mammary gland	(47)	(46)	(48)	(45)	(42)
Autolysis					1 (2%)
Cyst		1 (2%)			
Fibrosis				1 (2%)	
Alveolus, hyperplasia		1 (2%)		2 (4%)	1 (2%)
Skin	(48)	(46)	(48)	(45)	(43)
Fat, necrosis				1 (2%)	
Inflammation, chronic active				1 (2%)	
Necrosis				1 (2%)	
Sebaceous gland, hyperkeratosis				1 (2%)	
Sebaceous gland, hyperplasia				1 (2%)	
Musculoskeletal System					
Bone, femur	(48)	(48)	(48)	(47)	(47)
Fibro-osseous lesion			1 (2%)		
Skeletal muscle	(47)	(46)	(48)	(45)	(42)
Degeneration				1 (2%)	1 (2%)
Infiltration cellular, lymphocyte			1 (2%)	1 (2%)	
Nervous System					
Brain, brain stem	(47)	(47)	(48)	(45)	(42)
Compression		1 (2%)		1 (2%)	
Hemorrhage	1 (2%)				
Infiltration cellular, mononuclear cell		1 (2%)			
Mineralization	31 (66%)	24 (51%)	33 (69%)	24 (53%)	16 (38%)
Brain, cerebellum	(47)	(47)	(48)	(45)	(41)
Infiltration cellular, lymphocyte		1 (2%)			
Infiltration cellular, mononuclear cell		1 (2%)			
Brain, cerebrum	(48)	(47)	(48)	(45)	(41)
Cyst epithelial inclusion	1 (2%)				
Gliosis		1 (2%)			
Hemorrhage	1 (2%)	1 (2%)			
Infiltration cellular, histiocyte					1 (2%)
Infiltration cellular, lymphocyte	1 (2%)				
Infiltration cellular, mononuclear cell	1 (2%)	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Malformation	1 (2%)				
Mineralization	24 (50%)	19 (40%)	19 (40%)	18 (40%)	9 (22%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Nervous System (continued)					
Brain, cerebrum					
Necrosis		1 (2%)			
Meninges, pigmentation	1 (2%)				1 (2%)
Meninges, perivascular, polyarteritis	1 (2%)				
Peripheral nerve, sciatic	(46)	(47)	(48)	(45)	(42)
Infiltration cellular, mononuclear cell	1 (2%)		3 (6%)	1 (2%)	1 (2%)
Axon, degeneration	24 (52%)	25 (53%)	24 (50%)	17 (38%)	15 (36%)
Nerve, degeneration	2 (4%)				
Schwann cell, hyperplasia		1 (2%)	3 (6%)	1 (2%)	1 (2%)
Spinal cord, cervical	(47)	(47)	(48)	(45)	(44)
Compression				1 (2%)	
Cyst				1 (2%)	
Demyelination	1 (2%)				
Gliosis	1 (2%)	1 (2%)			
Infiltration cellular, lymphocyte	1 (2%)				
Infiltration cellular, mononuclear cell		2 (4%)		1 (2%)	
Axon, degeneration	4 (9%)	4 (9%)	6 (13%)	8 (18%)	3 (7%)
Spinal cord, lumbar	(47)	(47)	(48)	(45)	(45)
Infiltration cellular, lymphocyte	1 (2%)				
Infiltration cellular, mononuclear cell	2 (4%)	5 (11%)	4 (8%)	2 (4%)	1 (2%)
Polyarteritis	1 (2%)				
Axon, degeneration	25 (53%)	25 (53%)	23 (48%)	21 (47%)	11 (24%)
Nerve, degeneration	38 (81%)	37 (79%)	38 (79%)	32 (71%)	32 (71%)
Spinal cord, thoracic	(48)	(47)	(48)	(45)	(44)
Cyst				1 (2%)	
Gliosis				1 (2%)	
Infiltration cellular, lymphocyte	1 (2%)				
Infiltration cellular, mononuclear cell		1 (2%)	1 (2%)	1 (2%)	
Polyarteritis	1 (2%)				
Axon, degeneration	41 (85%)	34 (72%)	34 (71%)	33 (73%)	22 (50%)
Respiratory System					
Lung	(47)	(47)	(48)	(45)	(45)
Autolysis					1 (2%)
Hemorrhage	1 (2%)		2 (4%)	1 (2%)	1 (2%)
Infiltration cellular, histiocyte	1 (2%)		3 (6%)	2 (4%)	3 (7%)
Infiltration cellular, lymphocyte	3 (6%)	3 (6%)	6 (13%)	2 (4%)	
Inflammation, chronic active				1 (2%)	1 (2%)
Mineralization			1 (2%)		
Polyarteritis	1 (2%)				
Thrombosis				1 (2%)	
Alveolar epithelium, hyperplasia	1 (2%)	2 (4%)	3 (6%)	1 (2%)	5 (11%)
Nose	(47)	(46)	(47)	(45)	(43)
Hyaline droplet	6 (13%)	2 (4%)	3 (6%)		
Inflammation, suppurative					1 (2%)
Mucosa, ulcer					1 (2%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Acrylamide (continued)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Special Senses System					
Eye	(45)	(44)	(47)	(45)	(38)
Cataract	2 (4%)	2 (5%)	7 (15%)	10 (22%)	11 (29%)
Inflammation, suppurative				1 (2%)	
Inflammation, chronic active					1 (3%)
Phthisis bulbi		1 (2%)	2 (4%)	3 (7%)	1 (3%)
Bilateral, cataract	1 (2%)			1 (2%)	2 (5%)
Cornea, degeneration					1 (3%)
Cornea, inflammation, suppurative				1 (2%)	
Cornea, inflammation, chronic active			3 (6%)	2 (4%)	3 (8%)
Cornea, ulcer			1 (2%)	2 (4%)	1 (3%)
Harderian gland	(45)	(44)	(48)	(47)	(43)
Autolysis				1 (2%)	
Cyst		1 (2%)	1 (2%)		
Hyperplasia					2 (5%)
Infiltration cellular, lymphocyte		2 (5%)		1 (2%)	1 (2%)
Acinus, degeneration			1 (2%)		
Urinary System					
Kidney	(47)	(46)	(48)	(45)	(40)
Autolysis	1 (2%)				
Cyst	1 (2%)				
Hyaline droplet		4 (9%)	2 (4%)	2 (4%)	2 (5%)
Infiltration cellular, lymphocyte	33 (70%)	25 (54%)	27 (56%)	15 (33%)	13 (33%)
Metaplasia, osseous	1 (2%)			2 (4%)	
Nephropathy	2 (4%)	1 (2%)	2 (4%)		5 (13%)
Pigmentation					1 (3%)
Thrombosis					1 (3%)
Glomerulus, amyloid deposition	2 (4%)	1 (2%)			
Glomerulus, inflammation, chronic		1 (2%)			
Ureter	(0)	(0)	(0)	(0)	(1)
Urinary bladder	(45)	(45)	(48)	(45)	(38)
Infiltration cellular, lymphocyte	25 (56%)	20 (44%)	23 (48%)	11 (24%)	13 (34%)
Polyarteritis	1 (2%)				
Lumen, dilatation			1 (2%)	1 (2%)	3 (8%)

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

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

TABLE E1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 2-Week Drinking Water Study of Acrylamide[a]

	0 mM	0.14 mM	0.35 mM	0.70 mM	1.41 mM	3.52 mM	7.03 mM
Male							
Necropsy body wt.	143.5 ± 6.5	154.4 ± 8.9	149.0 ± 9.3	146.7 ± 9.8	137.3 ± 8.0	131.1 ± 7.2	89.7 ± 10.7[b,*]
Brain							
Absolute	1.66 ± 0.06	1.76 ± 0.03	1.72 ± 0.02	1.74 ± 0.03	1.65 ± 0.04	1.61 ± 0.03	1.53 ± 0.06[b]
Liver							
Absolute	6.75 ± 0.35	7.73 ± 0.33	7.14 ± 0.37	7.28 ± 0.67	6.67 ± 0.50	6.40 ± 0.21	3.75 ± 0.36[b,*]
Relative[c]	4.70 ± 0.05	5.02 ± 0.11	4.80 ± 0.07	4.94 ± 0.16	4.85 ± 0.18	4.90 ± 0.12	4.21 ± 0.15[b]
Relative[d]	4.06 ± 0.12	4.39 ± 0.16	4.15 ± 0.19	4.16 ± 0.32	4.06 ± 0.40	3.99 ± 0.15	2.44 ± 0.15[b,*]
Female							
Necropsy body wt.	118.5 ± 2.2	117.4 ± 4.7	111.8 ± 3.5	109.9 ± 7.9	111.3 ± 1.1[b]	101.1 ± 3.8	79.0 ± 3.6*
Brain							
Absolute	1.66 ± 0.04	1.65 ± 0.03	1.68 ± 0.01	1.60 ± 0.08	1.65 ± 0.05[b]	1.52 ± 0.03	1.44 ± 0.03*
Liver							
Absolute	5.45 ± 0.16	5.56 ± 0.13	5.24 ± 0.13	4.99 ± 0.33	4.87 ± 0.07[b]	4.44 ± 0.20*	3.56 ± 0.19*
Relative[c]	4.60 ± 0.12	4.75 ± 0.13	4.71 ± 0.22	4.55 ± 0.07	4.38 ± 0.11[b]	4.40 ± 0.12	4.51 ± 0.06
Relative[d]	3.29 ± 0.15	3.38 ± 0.08	3.12 ± 0.08	3.12 ± 0.11	2.95 ± 0.12[b]	2.92 ± 0.09	2.48 ± 0.11*

[a] The data are presented in grams as the mean ± s.e.m. An asterisk (*) denotes significant difference ($p < 0.05$) from the control.
[b] Data based upon three rats only
[c] Liver weight/body weight × 100
[d] Liver weight/brain weight

TABLE E2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 2-Week Feed Study of Acrylamide[a]

	0 mg/kg	7.4 mg/kg	18.5 mg/kg	37 mg/kg	74 mg/kg	185 mg/kg	370 mg/kg
Male							
Necropsy body wt.	125.8 ± 6.6	121.0 ± 13.8	120.7 ± 11.0	124.6 ± 5.9	115.9 ± 4.6	111.4 ± 5.8	90.3 ± 2.9*
Brain							
Absolute	1.65 ± 0.02	1.63 ± 0.04	1.68 ± 0.05	1.68 ± 0.04	1.61 ± 0.02	1.57 ± 0.04	1.51 ± 0.03
Liver							
Absolute	6.56 ± 0.28	6.20 ± 0.72	6.31 ± 0.67	6.51 ± 0.28	6.06 ± 0.20	5.84 ± 0.39	4.58 ± 0.23*
Relative[b]	5.23 ± 0.12	5.13 ± 0.12	5.20 ± 0.08	5.24 ± 0.07	5.24 ± 0.08	5.24 ± 0.11	5.06 ± 0.12
Relative[c]	3.98 ± 0.12	3.79 ± 0.35	3.73 ± 0.32	3.88 ± 0.16	3.75 ± 0.09	3.71 ± 0.16	3.03 ± 0.10
Female							
Necropsy body wt.	96.3 ± 4.6	96.6 ± 9.2	99.3 ± 5.6	98.3 ± 3.6	98.6 ± 5.3	97.1 ± 3.7	81.4 ± 2.9
Brain							
Absolute	1.51 ± 0.04	1.58 ± 0.05	1.59 ± 0.02	1.55 ± 0.04	1.57 ± 0.02	1.52 ± 0.02	1.40 ± 0.02
Liver							
Absolute	4.73 ± 0.35	5.23 ± 0.34	4.80 ± 0.40	4.96 ± 0.14	4.77 ± 0.18	4.87 ± 0.23	3.84 ± 0.09
Relative[b]	4.90 ± 0.15	5.46 ± 0.20	4.82 ± 0.23	5.06 ± 0.15	4.85 ± 0.14	5.02 ± 0.15	4.73 ± 0.14
Relative[c]	3.11 ± 0.14	3.31 ± 0.14	3.01 ± 0.22	3.20 ± 0.07	3.04 ± 0.12	3.21 ± 0.13	2.74 ± 0.04

[a] The data are presented in grams as the mean ± s.e.m. An asterisk (*) denotes significant difference ($p < 0.05$) from the control.
[b] Liver weight/body weight × 100
[c] Liver weight/brain weight

TABLE E3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Drinking Water Study of Acrylamide[a]

	0 mM	0.14 mM	0.35 mM	0.70 mM	1.41 mM	3.52 mM
Male						
Necropsy body wt.	319.7 ± 7.2	331.7 ± 4.8	320.9 ± 5.8	321.4 ± 6.2	306.1 ± 6.1	226.9 ± 4.2*
Brain						
Absolute	1.99 ± 0.03	1.97 ± 0.03	2.00 ± 0.02	1.96 ± 0.01	1.93 ± 0.02	1.81 ± 0.01*
Liver						
Absolute	9.69 ± 0.37	9.90 ± 0.32	9.54 ± 0.33	9.94 ± 0.23	10.15 ± 0.34	7.47± 0.22*
Relative[b]	3.03 ± 0.06	2.98 ± 0.07	2.97 ± 0.07	3.10 ± 0.05	3.33 ± 0.13	3.29 ± 0.06*
Relative[c]	4.87 ± 0.19	5.04 ± 0.15	4.78 ± 0.19	5.09 ± 0.11	5.27 ± 0.19	4.12 ± 0.13*
Female						
Necropsy body wt.	191.3 ± 2.5	195.1 ± 2.7	186.1 ± 3.4	185.7 ± 1.8	172.8 ± 7.1*	132.9 ± 3.7*
Brain						
Absolute	1.81 ± 0.02	1.87 ± 0.02	1.78 ± 0.04	1.83 ± 0.02	1.79 ± 0.02	1.63 ± 0.02*
Liver						
Absolute	5.13 ± 0.15	5.09 ± 0.15	5.07 ± 0.14	5.22 ± 0.16	4.90 ± 0.26	4.79 ± 0.17
Relative[b]	2.68 ± 0.06	2.61 ± 0.07	2.72 ± 0.06	2.81 ± 0.07	2.83 ± 0.08	3.61 ± 0.09*
Relative[c]	2.83 ± 0.08	2.72 ± 0.07	2.84 ± 0.05	2.85 ± 0.08	2.74 ± 0.13	2.94 ± 0.10

[a] The data are presented in grams as the mean ± s.e.m. An asterisk (*) denotes significant difference ($p < 0.05$) from the control.
[b] Liver weight/body weight × 100
[c] Liver weight/brain weight

TABLE E4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Feed Study of Acrylamide[a]

	0 mg/kg	7.4 mg/kg	18.5 mg/kg	37 mg/kg	74 mg/kg	185 mg/kg
Male						
Necropsy body wt.	330.3 ± 4.4	334.8 ± 6.4	320.3 ± 5.4	329.4 ± 4.5	325.6 ± 9.0	282.0 ± 7.2*
Brain						
Absolute	1.96 ± 0.02	1.99 ± 0.03	1.94 ± 0.03	2.00 ± 0.03	1.96 ± 0.03	1.88 ± 0.03
Liver						
Absolute	8.97 ± 0.21	8.88 ± 0.20	8.81 ± 0.25	8.89 ± 0.21	8.88 ± 0.38	8.78 ± 0.38
Relative[b]	2.72 ± 0.04	2.65 ± 0.02	2.75 ± 0.06	2.70 ± 0.04	2.72 ± 0.06	3.11 ± 0.06*
Relative[c]	4.58 ± 0.12	4.45 ± 0.09	4.54 ± 0.15	4.45 ± 0.10	4.53 ± 0.14	4.67 ± 0.19
Female						
Necropsy body wt.	195.2 ± 4.2	194.9 ± 3.0	188.9 ± 4.2	190.3 ± 2.3	183.4 ± 2.8	156.5 ± 4.5*
Brain						
Absolute	1.80 ± 0.03	1.85 ± 0.03	1.81 ± 0.03	1.79 ± 0.04	1.82 ± 0.03	1.66 ± 0.03*
Liver						
Absolute	5.12 ± 0.21	5.12 ± 0.15	4.89 ± 0.15	4.92 ± 0.11	4.79 ± 0.17	4.16 ± 0.14*
Relative[b]	2.62 ± 0.08	2.63 ± 0.07	2.58 ± 0.07	2.59 ± 0.07	2.61 ± 0.07	2.66 ± 0.04
Relative[c]	2.86 ± 0.15	2.77 ± 0.10	2.69 ± 0.09	2.76 ± 0.10	2.63 ± 0.08	2.52 ± 0.10

[a] The data are presented in grams as the mean ± s.e.m. An asterisk (*) denotes significant difference ($p < 0.05$) from the control.
[b] Liver weight/body weight × 100
[c] Liver weight/brain weight

TABLE E5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 2-Week Drinking Water Study of Acrylamide

	0 mM	0.14 mM	0.35 mM	0.70 mM	1.41 mM	3.52 mM	7.03 mM
Male							
Necropsy body wt.[a]	17.3 ± 0.4	17.4 ± 0.2	16.5 ± 0.2	18.5 ± 0.3	18.1 ± 0.6	18.6 ± 0.5	-
Brain							
Absolute[b]	434 ± 10	431 ± 2	429 ± 3	434 ± 11	434 ± 8	413 ± 5	-
Liver							
Absolute	737 ± 14	823 ± 31	678 ± 32	885 ± 23*	827 ± 29	819 ± 10	-
Relative[c]	4.27 ± 0.07	4.72 ± 0.12	4.11 ± 0.20	4.78 ± 0.09*	4.57 ± 0.07	4.41 ± 0.08	-
Relative[d]	1.71 ± 0.04	1.91 ± 0.07	1.58 ± 0.07	2.05 ± 0.05*	1.91 ± 0.07	1.98 ± 0.02*	-
Female							
Necropsy body wt.	14.6 ± 0.5	15.0 ± 0.6	14.7 ± 0.3	14.4 ± 0.3	14.8 ± 0.5	14.7 ± 0.7	-
Brain							
Absolute	416 ± 11	428 ± 4	428 ± 5	421 ± 8	427 ± 12	399 ± 13	-
Liver							
Absolute	572 ± 29	646 ± 40	605 ± 21	615 ± 14	657 ± 20	626 ± 19	-
Relative[c]	3.92 ± 0.12	4.30 ± 0.12	4.13 ± 0.11	4.28 ± 0.03	4.47 ± 0.24	4.26 ± 0.11	-
Relative[d]	1.38 ± 0.07	1.51 ± 0.09	1.41 ± 0.05	1.46 ± 0.03	1.54 ± 0.04	1.57 ± 0.06	-

[a] Body weight data are presented in grams as the mean ± s.e.m. An asterisk (*) denotes significant difference ($p < 0.05$) from the control.
[b] Organ weight data are presented in milligrams as the mean ± s.e.m. An asterisk (*) denotes significant difference ($p < 0.05$) from the control.
[c] Liver weight/body weight × 100
[d] Liver weight/brain weight

TABLE E6
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 2-Week Feed Study of Acrylamide

	0 mg/kg	18.5 mg/kg	37 mg/kg	74 mg/kg	185 mg/kg	370 mg/kg	370 mg/kg
Male							
Necropsy body wt.[a]	18.2 ± 0.5	18.8 ± 0.4	19.3 ± 0.2	19.0 ± 0.5	17.8 ± 0.5	18.6 ± 0.3	17.0 ± 0.7
Brain							
Absolute[b]	433 ± 6	442 ± 4	431 ± 8	435 ± 5	422 ± 5	432 ± 6	414 ± 9
Liver							
Absolute	761 ± 16	825 ± 32	884 ± 34	839 ± 32	786 ± 28	835 ± 13	717 ± 63
Relative[c]	4.19 ± 0.06	4.39 ± 0.08	4.58 ± 0.15	4.41 ± 0.06	4.42 ± 0.13	4.50 ± 0.10	4.20 ± 0.23
Relative[d]	1.76 ± 0.04	1.87 ± 0.09	2.05 ± 0.10	1.93 ± 0.06	1.86 ± 0.07	1.94 ± 0.06	1.73 ± 0.14
Female							
Necropsy body wt.	15.2 ± 0.5	15.4 ± 0.6	14.7 ± 0.7	14.6 ± 0.2	15.0 ± 0.1	14.4 ± 0.8	14.1 ± 0.6
Brain							
Absolute	428 ± 13	411 ± 15	436 ± 10	441 ± 17	432 ± 6	428 ± 9	402 ± 5
Liver							
Absolute	674 ± 37	697 ± 23[e]	625 ± 46	665 ± 20	645 ± 30	615 ± 35	597 ± 42
Relative[c]	4.44 ± 0.12	4.59 ± 0.10[e]	4.25 ± 0.13	4.54 ± 0.07	4.31 ± 0.18	4.28 ± 0.07	4.23 ± 0.16
Relative[d]	1.58 ± 0.07	1.68 ± 0.13[e]	1.44 ± 0.11	1.51 ± 0.04	1.50 ± 0.07	1.44 ± 0.07	1.49 ± 0.09

[a] Body weight data are presented in grams as the mean ± s.e.m. An asterisk (*) denotes significant difference ($p < 0.05$) from the control.
[b] Organ weight data are presented in milligrams as the mean ± s.e.m. An asterisk (*) denotes significant difference ($p < 0.05$) from the control.
[c] Liver weight/body weight × 100
[d] Liver weight/brain weight
[e] Data based upon three mice only

TABLE E7
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Drinking Water Study of Acrylamide[a]

	0 mM	0.14 mM	0.35 mM	0.70 mM	1.41 mM	3.52 mM
Male						
Necropsy body wt.	26.8 ± 0.7	24.3 ± 0.4*	25.8 ± 0.5	24.5 ± 0.4*	24.4 ± 0.3*	23.4 ± 0.9*
Brain						
Absolute	0.46 ± 0.009	0.47 ± 0.006	0.47 ± 0.004	0.46 ± 0.006	0.47 ± 0.008	0.43 ± 0.006*
Liver						
Absolute	1.02 ± 0.03	0.93 ± 0.02	1.05 ± 0.03	0.96 ± 0.01	1.14 ± 0.03*	0.97 ± 0.04
Relative[b]	3.81 ± 0.13	3.84 ± 0.07	4.08 ± 0.07	3.91 ± 0.06	4.69 ± 0.16*	4.17 ± 0.08
Relative[c]	2.21 ± 0.07	2.00 ± 0.05	2.32 ± 0.07	2.07 ± 0.04	2.42 ± 0.05	2.28 ± 0.10
Female						
Necropsy body wt.	21.0 ± 0.7	21.2 ± 0.3	21.0 ± 0.3	20.6 ± 0.3	20.9 ± 0.5	18.5 ± 0.2*
Brain						
Absolute	0.48 ± 0.008	0.49 ± 0.007	0.48 ± 0.008	0.47 ± 0.006	0.46 ± 0.003	0.42 ± 0.007*
Liver						
Absolute	0.86 ± 0.03	0.93 ± 0.02	0.93 ± 0.02	0.90 ± 0.02	0.88 ± 0.02	0.82 ± 0.02
Relative[b]	4.13 ± 0.16	4.39 ± 0.06	4.44 ± 0.08	4.39 ± 0.09	4.22 ± 0.07	4.45 ± 0.09
Relative[c]	1.79 ± 0.05	1.89 ± 0.04	1.95 ± 0.02	1.92 ± 0.05	1.89 ± 0.04	1.97 ± 0.06

[a] The data are presented in grams as the mean ± s.e.m. An asterisk (*) denotes significant difference ($p < 0.05$) from the control.
[b] Liver weight/body weight × 100
[c] Liver weight/brain weight

TABLE E8
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Feed Study of Acrylamide[a]

	0 mg/kg	18.5 mg/kg[b]	37 mg/kg	74 mg/kg	185 mg/kg[b]	370 mg/kg[b]
Male						
Necropsy body wt.	24.9 ± 0.5	26.8 ± 0.7	25.5 ± 0.7	25.2 ± 0.6	25.1 ± 0.6	22.0 ± 0.9*
Brain						
Absolute	0.46 ± 0.01	0.46 ± 0.01	0.45 ± 0.02	0.45 ± 0.009	0.44 ± 0.01	0.40 ± 0.009*
Liver						
Absolute	1.17 ± 0.03	1.22 ± 0.06	1.18 ± 0.04	1.15 ± 0.03	1.17 ± 0.02	0.98 ± 0.03*
Relative[c]	4.71 ± 0.11	4.56 ± 0.16	4.62 ± 0.07	4.56 ± 0.06	4.68 ± 0.04	4.47 ± 0.15
Relative[d]	2.56 ± 0.12	2.68 ± 0.12	2.63 ± 0.07	2.54 ± 0.07	2.67 ± 0.08	2.43 ± 0.05
Female						
Necropsy body wt.	21.0 ± 0.3	21.6 ± 0.3	20.5 ± 0.3	20.3 ± 0.4	20.5 ± 0.5	16.4 ± 0.4*
Brain						
Absolute	0.47 ± 0.006	0.47 ± 0.01	0.46 ± 0.007	0.46 ± 0.009	0.44 ± 0.008	0.40 ± 0.007*
Liver						
Absolute	0.94 ± 0.03	0.95 ± 0.03	0.92 ± 0.03	0.92 ± 0.03	0.94 ± 0.02	0.76 ± 0.02*
Relative[c]	4.49 ± 0.07	4.41 ± 0.12	4.51 ± 0.11	4.55 ± 0.11	4.61 ± 0.07	4.65 ± 0.06
Relative[d]	2.01 ± 0.07	2.03 ± 0.05	2.00 ± 0.06	2.00 ± 0.05	2.14 ± 0.05	1.92 ± 0.04

[a] The data are presented in grams as the mean ± s.e.m. An asterisk (*) denotes significant difference ($p < 0.05$) from the control.
[b] Data based upon seven mice only
[c] Liver weight/body weight × 100
[d] Liver weight/brain weight

APPENDIX F
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF ACRYLAMIDE

The acrylamide test article used was purchased from Sigma Chemical Company of St. Louis, Missouri (Lot 102K0162) and received March 14, 2003. The compound was stored in its original glass bottle and cardboard box in a locked cabinet. Identity, purity, and stability analyses were conducted by the Division of Biochemical Toxicology Chemistry Support Group (DBT/CHEM) at the National Center for Toxicological Research (NCTR; Jefferson, AR). Reports on analyses performed in support of the acrylamide studies are on file at NCTR.

Test sample characterization was performed using gas chromatography with electron impact mass spectrometry (GC/EI-MS; MS model TSQ 700; DB-5 capillary GC column). The sample (1.4 mg) was dissolved with 1 ml of methanol and diluted 10x with methanol. Initial temperature (75°C) was adjusted for sufficient separation from the methanol solvent peak. The final analysis was performed with the strongest concentration sample. Based on the library reference spectrum, the major component (t_r = 3.76 min; m/z 71) was tentatively identified as acrylamide (CAS# 76-06-1). Based on integration of the RIC peaks for components not related to column bleed, the purity of the sample was 99.4%.

Proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra of the acrylamide samples (Sigma Lot 102K0162) were recorded with deuterated methanol as solvent (36 mg/ml for ^{1}H NMR and 194 mg/ml for ^{13}C NMR). The ^{1}H and ^{13}C NMR chemical shifts and coupling patterns were consistent with the acrylamide structure (Figures F1 and F2). Additionally, two small broad proton resonances (7.70 and 6.98 ppm) from the NH_2 group were detected in the expanded ^{1}H NMR spectrum. These arise from residual exchangeable protons that were present even though deuterated methanol was used as solvent. The acrylamide samples were very pure with respect to proton containing organics and were estimated at greater than 99.9% purity.

Purity analysis of samples of acrylamide Sigma Lot 102K0162 was determined by capillary gas chromatography with flame ionization detection (GC/FID). For analysis of dosed water, the GC instrument was a Hewlett Packard HP 6980A operated in the capillary split mode (1:10) using a 320μm diameter, 30 m length, 0.25 μm film thickness fused silica capillary J%W DB-1701, and the oven programmed from 35° C (0.2 min hold) to 200°C (2 min final hold). The capillary inlet and FID detector temperatures were held at 200° C and 250° C, respectively. One microliter injections of 2 mg/ml solutions of the test compounds in EtOAc were made and compared to EtOAc solvent blank using GC/FID analysis. For analysis of dosed feed, the GC set up was the same, except for the 0.5 μm film thickness fused silica Supelco PTA-5 capillary column and the GC oven programmed at 30°/min from 40° C (0.5 min initial hold) to 280° C (no final hold). Acrylamide was analyzed for purity based on the percentage of total area observed by GC/FID response. No impurity peaks (<0.1%) were evident with acrylamide elution at 10.5 min, indicating a purity of >99.9% (Figure F3). Samples of acrylamide Sigma Lot 102K0162 were analyzed for purity approximately every six months until the end of the study. Each evaluation including the end-of-study analysis indicated that no change had occurred in Sigma Lot 102K0162 during the course of study.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by dissolving acrylamide in water to give the required concentrations (Table F1). The dose formulations were stored in capped bottles protected from light at room temperature.

A homogeneity study of dosed feed at a concentration of 37 ppm acrylamide and a stability study of a 10 μg/ml acrylamide formulation were performed using GC-FID as described above. Stability was confirmed for at least 49 days for dose formulations stored in capped bottles at room temperature with protection from light.

Periodic analyses of the dose formulations of acrylamide were conducted using GC-FID. Dose formulations were analyzed twice during the 2-week studies (Table F2), approximately every 2 weeks during the 3-month studies (Table F3), and approximately every 2 to 3 months during the 2-year studies (Table F4). Of the dose formulations

analyzed and used during the 2-week studies, 14 of 19 were within 10% of the target concentrations. Of the dose formulations analyzed and used during the 3-month studies, 56 of 67 were within 10% of the target concentrations. Of the dose formulations analyzed and used during the 2-year studies, all 52 for rats and mice were within 10% of the target concentrations.

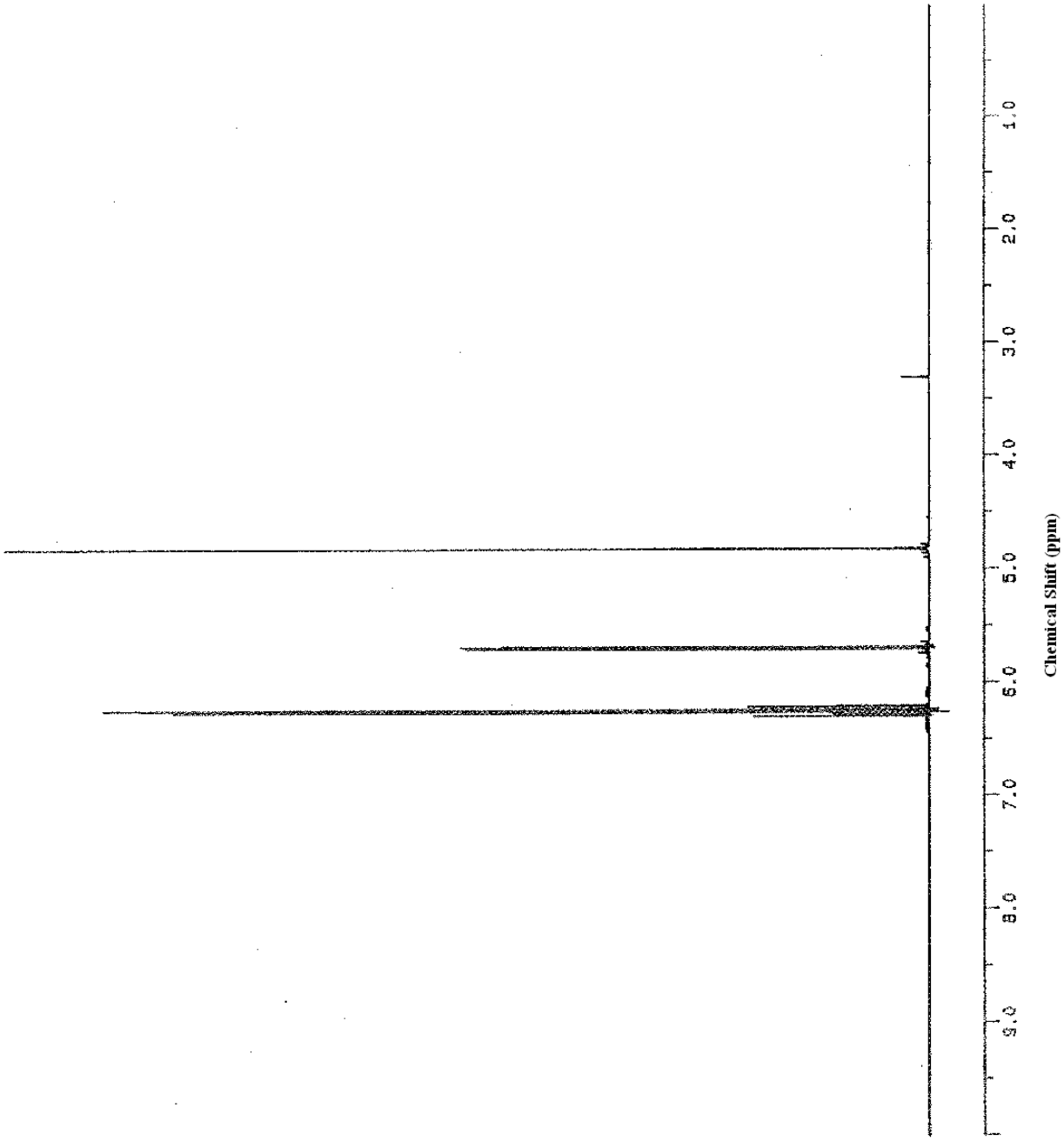

FIGURE F1
Proton Nuclear Magnetic Resonance Spectrum of Acrylamide

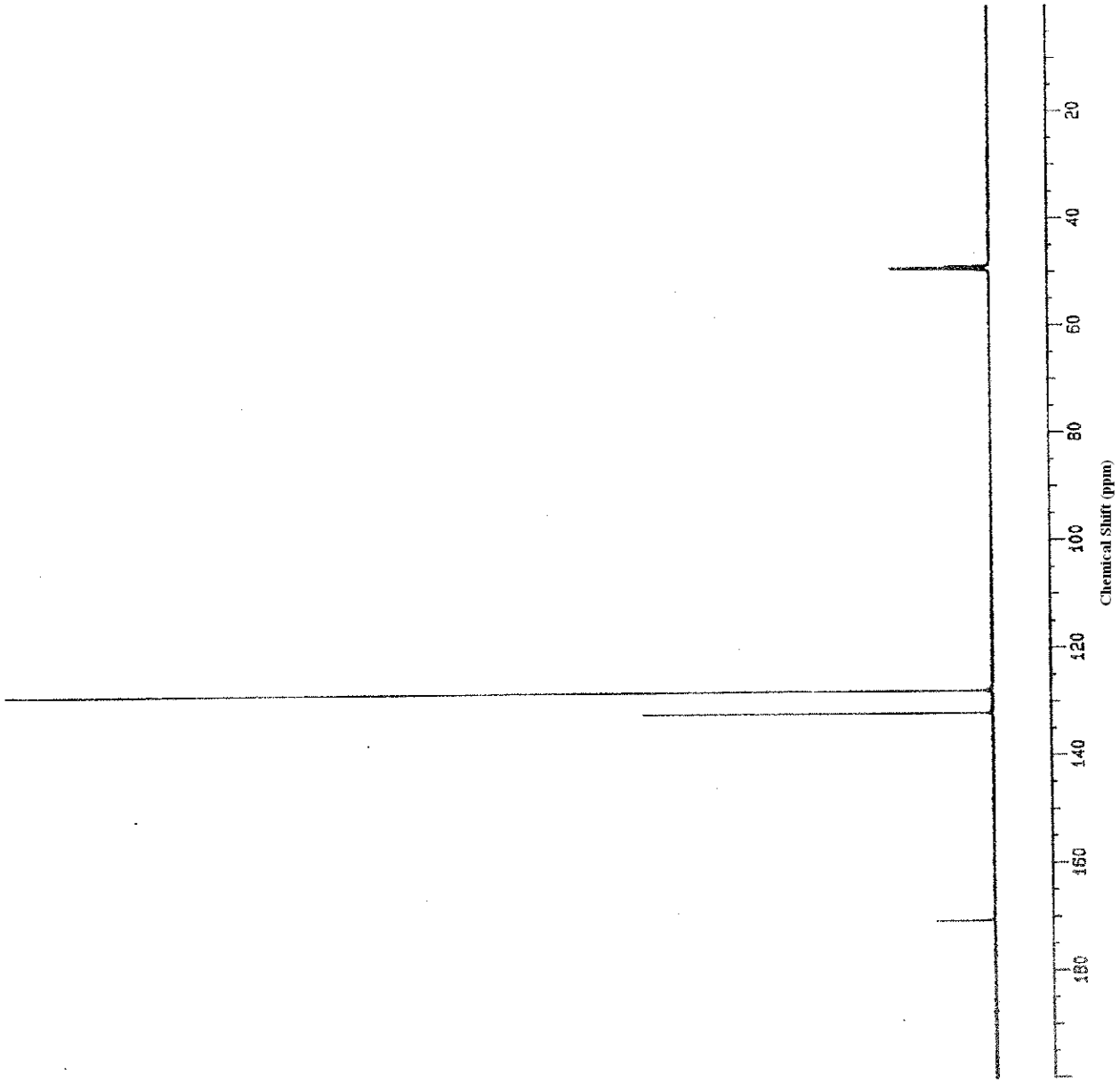

FIGURE F2
Carbon Nuclear Magnetic Resonance Spectrum of Acrylamide

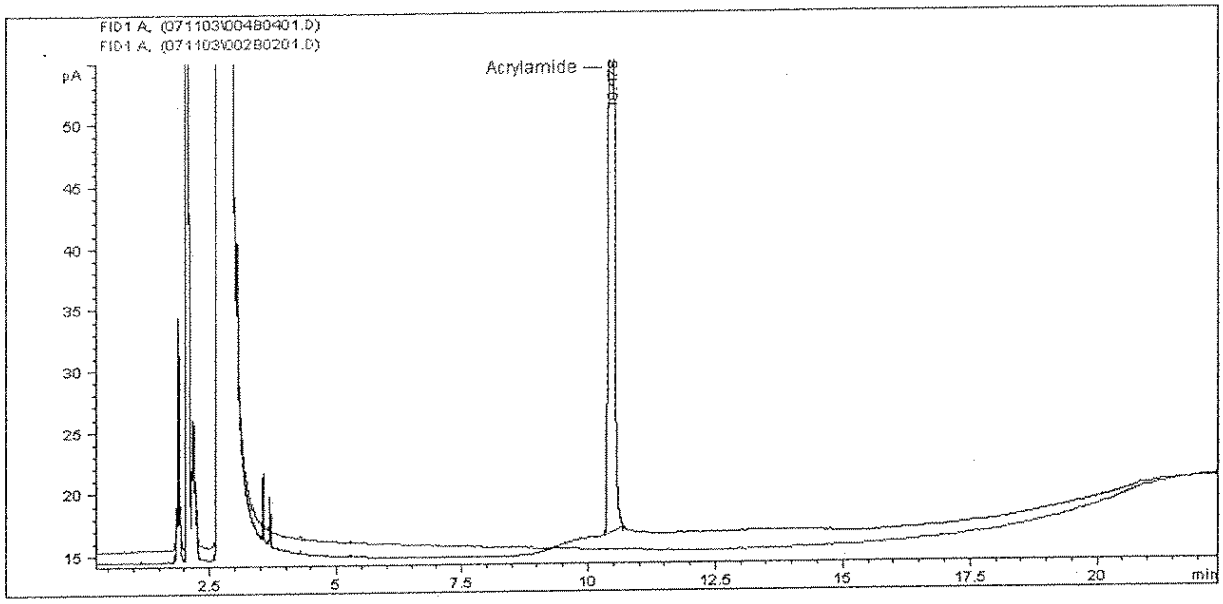

FIGURE F3
Capillary Gas Chromatography with Flame Ionization Detection Purity Analysis of Acrylamide

TABLE F1
Preparation and Storage of Dose Formulations in the Drinking Water and Feed Studies of Acrylamide

2-Week Studies	3-Month Studies	2-Year Studies
Preparation		
A stock solution of acrylamide (5 mg/ml) in water was prepared in a volumetric flask. The stock solution was diluted with Millipore filtered water to the needed concentrations. The dose formulations in drinking water were prepared weekly. NIH-31 IR Rodent diet was mixed for 10 min with an acrylamide solution using a 1.0 cu. ft. Patterson Kelley V blender with intensifier bar running. The dosed feed was prepared once.	A stock solution of acrylamide (5 mg/ml) in water was prepared in a volumetric flask. The stock solution was diluted with Millipore filtered water to the needed concentrations. The dose formulations in drinking water were prepared approximately every 2 weeks. NIH-31 IR Rodent diet was mixed for 10 min with an acrylamide solution using a 1.0 cu. ft. Patterson Kelley V blender with intensifier bar running. The dosed feed was prepared approximately every 2 weeks.	A stock solution of acrylamide (5 mg/ml) in water was prepared in a volumetric flask. The stock solution was diluted with Millipore filtered water to the needed concentrations. The dose formulations were prepared weekly.
Chemical Lot Number		
102K0162	102K0162	102K0162
Storage Conditions		
Dosed water was stored at 4° C with protection from light. Dosed feed was stored at 10° to 20° C.	Dosed water was stored at 4° C with protection from light. Dosed feed was stored at 10° to 20° C.	Dosed water was stored at 4° C with protection from light.
Study Laboratory		
National Center for Toxicological Research (Jefferson, Arkansas)	National Center for Toxicological Research (Jefferson, Arkansas)	National Center for Toxicological Research (Jefferson, Arkansas)

TABLE F2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Week Drinking Water and Feed Studies of Acrylamide

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
Drinking Water Samples				
March 31, 2004	July 12, 2004	0	<LOQ[b]	---
		10	8.7	-13
		25	23.3	-7
		50	47.4	-5
		100	101	+1
		250	250	0
		500	527	+5
April 7, 2004	July 14, 2004	0	<LOQ	---
		10	9.0	-10
		25	22.3	-11
		50	44.7	-10
		100	98.2	-2
		250	273	+9
		500	525	+5
Feed Samples				
April 14, 2004	July 21, 2004	0	<LOQ	---
		7.4	6.6	-11
		18.5	18.5	0
		37.0	33.3 ± 3.3[c]	-10
		74.0	65.6	-11
		185	177	-4
		370	339	-8
		37.0	33.3 ± 3.3[c]	-10

[a] Dosed water and feed were analyzed in duplicate and the average is reported.
[b] Limit of quantitation determined by GC-FID was 2 ppm.
[c] Based on analysis of n=9

TABLE F3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Drinking Water and Feed Studies of Acrylamide

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
Drinking Water Samples				
July 15, 2004	July 28, 2004	0	<LOQ[b]	---
		10	8.7	-13
		25	22.8	-9
		50	47.4	-5
		100	98.1	-2
		250	268	+7
August 3, 2004	August 13, 2004	0	<LOQ	---
		10	8.0	-20
		25	23.8	-5
		50	47.5	-5
		100	102	+2
		250	257	+3
August 19, 2004	August 25, 2004	0	<LOQ	---
		10	10.8	+8
		25	24.8	-1
		50	49.6	-1
		100	94.9	-5
		250	276	+10
September 3, 2004	September 18, 2004	0	<LOQ	---
		10	8.3	-17
		25	23.3	-7
		50	46.2	-8
		100	99.5	-1
		250	228	-9
September 17, 2004	October 5, 2004	0	<LOQ	---
		10	9.73	-3
		25	22.8	-9
		50	48.1	-4
		100	97.7	-2
		250	237	-5
October 1, 2004	October 18, 2004	0	<LOQ	---
		10	9.54	-5
		25	25.2	+1
		50	48.6	-3
		100	96.3	-4
		250	247	-1
Feed Samples				
August 11, 2004	August 30, 2004	0	<LOQ[c]	---
		7.4	7.6[d]	+2
		18.5	19.5	+6
		37.0	37.2	0
		74.0	71.7	-3
		185	188[e]	+1
		370	356	-4
August 30, 2004	September 18, 2004	0	<LOQ	---
		7.4	6.47	-13
		18.5	16.8	-9
		37.0	38.6	+4
		74.0	80.4	+9
		185	183	-1
		370	376	+2

TABLE F3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Drinking Water and Feed Studies of Acrylamide (continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
Feed Samples (continued)				
September 15, 2004	September 27, 2004	0	<LOQ	---
		7.4	7.33	-1
		18.5	18.4	-1
		37.0	36.9	0
		74.0	73.6	-1
		185	175	-5
September 23, 2004	October 15, 2004	0	<LOQ	---
		0	<LOQ	---
		370	758[e,f]	+105
September 29, 2004	October 13, 2004	0	<LOQ	---
		7.4	5.93[e]	-20
		18.5	16.1[e]	-13
		185	188[e]	+2
		370	339	-8
September 29, 2004	October 15, 2004	18.5	16.9	-9
		37.0	35.8	-3
		37.0	32.9	-11
		74.0	89.4[e]	+21
		74.0	68.1	-8
October 14, 2004	November 5, 2004	0	<LOQ	---
		7.4	6.22	-16
		18.5	17.9	-3
		37.0	37.6	+2
		74.0	77.2	+4
		185	185	0
October 22, 2004	November 5, 2004	0	<LOQ	---
		7.4	7.04	-5
		18.5	17.9	-3
		37.0	33.5	-9
		74.0	73.5	-1
		185	185	0
		370	341	-8

[a] Dosed water was analyzed in duplicate and the average is reported.
[b] Limit of quantitation determined by GC-FID was 0.2 µg/ml.
[c] Limit of quantitation determined by GC-FID was 0.26 µg/g.
[d] Based on a single sample analysis
[e] Based on analysis of n=4
[f] Batch not fed to animals

TABLE F4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Drinking Water Studies of Acrylamide

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
May 24, 2005	May 27, 2005	0	<LOQ[b]	---
		6.25	6.35	+2
		12.5	12.1	-3
		25.0	22.7	-9
		50.0	45.0	-10
July 26, 2005	August 1, 2005	0	<LOQ[c]	---
		6.25	5.63	-10
		12.5	12.0	-4
		25.0	23.4	-6
		50.0	45.7	-8
October 4, 2005	October 7, 2005	0	<LOQ	---
		6.25	5.72	-8
		12.5	12.2	-2
		25.0	25.2	+1
		50.0	48.1	-4
November 29, 2005	December 2, 2005	0	<LOQ	---
		6.25	6.34	+2
		12.5	12.2	-3
		25.0	24.3	-3
		50.0	50.2	0
February 7, 2006	February 8, 2006	0	<LOQ	---
		6.25	6.80	+9
		12.5	13.2	+6
		25.0	26.0	+4
		50.0	50.5	+1
April 18, 2006	April 21, 2006	0	<LOQ	---
		6.25	5.94	-5
		12.5	12.7	+1
		25.0	22.8	-9
		50.0	48.9	-2
June 20, 2006	June 26, 2006	0	<LOQ	---
		6.25	6.18	-1
		12.5	12.4	-1
		25.0	26.3	+5
		50.0	49.9	0
August 30, 2006	September 1, 2006	0	<LOQ	---
		6.25	6.07	-3
		12.5	12.0	-4
		25.0	24.9	0
		50.0	49.6	-1
November 7, 2006	November 14, 2006	0	<LOQ	---
		6.25	6.65	+6
		12.5	12.3	-2
		25.0	24.6	-2
		50.0	49.5	-1
January 9, 2007	January 11, 2007	0	<LOQ	---
		6.25	6.16	-1
		12.5	13.2	+6
		25.0	24.5	-2
		50.0	46.0	-8
March 20, 2007	March 23, 2007	0	<LOQ	---
		6.25	6.15	-2
		12.5	12.5	0
		25.0	25.2	+1
		50.0	46.4	-7
May 29, 2007	June 4, 2007	0	<LOQ	---
		6.25	6.10	-2
		12.5	11.8	-6
		25.0	22.7	-9
		50.0	46.2	-7

TABLE F4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Drinking Water Studies of Acrylamide (continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
July 31, 2007	August 3, 2007	0	<LOQ	---
		6.25	6.47	+4
		12.5	11.5	-8
		25.0	23.2	-7
		50.0	50.3[d]	+1

[a] Dosed water was analyzed in duplicate and the average is reported.
[b] Limit of quantitation determined by GC-FID was 0.2 µg/ml.
[c] Sample also analyzed by LC-MS and acrylamide determined at <0.005 µg/ml
[d] Based on a single sample analysis

APPENDIX G
FOOD CONSUMPTION IN THE 2-YEAR DRINKING WATER STUDY OF ACRYLAMIDE

TABLE G1
Food Consumption of Male Rats in the 2-Year Drinking Water Study of Acrylamide

Week[a]	0.0 mM			0.0875 mM				0.175 mM				0.35 mM				0.70 mM			
	N[b]	Mean ± SE[c]	P-Value[d]	N	Mean ± SE	Pct[e]	P-Value	N	Mean ± SE	Pct	P-Value	N	Mean ± SE	Pct	P-Value	N	Mean ± SE	Pct	P-Value
4	24	15.1 ± 0.2	**0.027**	24	15.1 ± 0.2	99.9	1.000	24	15.2 ± 0.2	100.3	0.999	24	15.2 ± 0.2	100.6	0.992	24	15.6 ± 0.2	103.2	0.167
8	24	16.5 ± 0.2	0.163	24	16.7 ± 0.2	101.0	0.965	24	16.5 ± 0.2	99.9	1.000	24	16.3 ± 0.2	98.4	0.826	24	17.0 ± 0.2	103.1	0.310
12	24	16.6 ± 0.2	0.889	24	16.5 ± 0.2	99.6	0.998	24	16.5 ± 0.2	99.7	0.999	24	16.2 ± 0.2	97.6	0.336	24	16.6 ± 0.2	100.5	0.994
16	24	16.6 ± 0.2	0.528	24	16.6 ± 0.2	99.8	1.000	24	16.6 ± 0.2	99.8	1.000	24	16.4 ± 0.2	99.0	0.964	24	16.8 ± 0.2	101.2	0.929
20	24	17.1 ± 0.2	0.270	24	16.7 ± 0.2	97.5	0.337	24	16.9 ± 0.2	98.4	0.718	24	16.4 ± 0.2	95.6	**0.022**	24	17.3 ± 0.2	101.2	0.885
24	24	17.1 ± 0.2	0.452	24	17.0 ± 0.2	99.2	0.942	24	17.2 ± 0.2	100.7	0.967	24	16.9 ± 0.2	98.7	0.782	24	17.3 ± 0.2	101.0	0.884
28	24	17.3 ± 0.2	**0.030**	24	17.1 ± 0.2	99.3	0.956	24	17.0 ± 0.2	98.6	0.638	24	17.1 ± 0.2	99.1	0.888	24	17.6 ± 0.2	102.0	0.286
32	24	17.2 ± 0.2	0.510	24	17.3 ± 0.2	100.5	0.980	24	17.2 ± 0.2	100.2	1.000	24	17.0 ± 0.2	99.0	0.835	24	17.4 ± 0.2	101.2	0.747
36	24	17.2 ± 0.2	0.698	24	17.1 ± 0.2	99.7	0.999	24	17.2 ± 0.2	100.2	1.000	24	16.7 ± 0.2	97.5	0.174	24	17.2 ± 0.2	100.0	1.000
40	24	17.0 ± 0.2	0.819	24	17.2 ± 0.2	101.2	0.850	24	16.9 ± 0.2	99.4	0.985	24	16.6 ± 0.2	97.9	0.504	24	17.2 ± 0.2	101.1	0.895
44	24	17.1 ± 0.2	0.904	24	17.5 ± 0.2	102.0	0.711	24	17.0 ± 0.2	99.1	0.977	24	17.1 ± 0.2	99.5	0.997	24	17.2 ± 0.2	100.5	0.997
48	24	18.0 ± 0.3	0.284	24	18.6 ± 0.3	103.0	0.602	24	18.0 ± 0.3	99.7	1.000	24	17.9 ± 0.3	99.1	0.992	24	17.8 ± 0.3	98.8	0.975
52	24	19.5 ± 0.4	0.777	24	20.3 ± 0.4	104.1	0.424	24	19.2 ± 0.4	98.2	0.925	24	19.0 ± 0.4	97.2	0.727	24	19.7 ± 0.4	101.1	0.987
56	24	20.2 ± 0.3	0.996	24	20.9 ± 0.3	103.4	0.269	24	20.6 ± 0.3	101.7	0.824	24	20.2 ± 0.3	99.8	1.000	24	20.6 ± 0.3	101.7	0.805
60	24	20.8 ± 0.3	0.540	24	21.2 ± 0.3	101.8	0.791	24	20.5 ± 0.3	98.7	0.915	24	19.9 ± 0.3	95.4	0.080	24	20.9 ± 0.3	100.3	1.000
64	24	20.7 ± 0.3	0.284	24	21.2 ± 0.3	102.7	0.596	24	20.6 ± 0.3	99.6	1.000	24	20.0 ± 0.3	96.9	0.474	23	20.5 ± 0.3	99.4	0.997
68	24	20.0 ± 0.4	0.799	24	21.9 ± 0.4	109.3	**0.004**	24	20.9 ± 0.4	104.2	0.358	24	20.5 ± 0.4	102.3	0.828	23	20.7 ± 0.4	103.5	0.547
72	24	20.7 ± 0.6	0.190	24	22.5 ± 0.6	108.8	0.089	24	21.7 ± 0.6	104.8	0.558	24	20.7 ± 0.6	100.0	1.000	23	20.7 ± 0.6	99.8	1.000
76	24	20.7 ± 0.5	0.191	24	22.5 ± 0.5	108.8	0.067	24	20.7 ± 0.5	100.1	1.000	24	20.8 ± 0.5	100.6	1.000	23	20.5 ± 0.6	99.0	0.996
80	24	21.7 ± 0.8	0.277	24	23.7 ± 0.8	109.5	0.211	24	21.4 ± 0.8	98.6	0.997	24	20.6 ± 0.8	95.1	0.759	23	21.5 ± 0.8	99.4	1.000
84	23	23.9 ± 2.7	0.312	24	30.2 ± 2.7	126.4	0.273	23	22.7 ± 2.7	95.0	0.993	23	20.2 ± 2.7	84.5	0.725	22	23.4 ± 2.7	98.2	1.000
88	22	22.7 ± 1.5	0.594	23	26.5 ± 1.5	117.1	0.205	22	22.9 ± 1.5	101.0	1.000	22	23.6 ± 1.5	104.1	0.979	21	25.1 ± 1.5	110.7	0.616
92	21	23.6 ± 1.1	0.097	22	25.8 ± 1.1	109.6	0.405	22	24.0 ± 1.1	102.1	0.993	22	20.5 ± 1.1	87.1	0.163	21	27.5 ± 1.1	116.8	**0.043**
96	21	22.8 ± 1.4	0.352	19	26.3 ± 1.5	115.2	0.263	21	24.3 ± 1.4	106.7	0.869	20	20.9 ± 1.4	91.6	0.752	20	26.6 ± 1.5	116.7	0.198
100	19	22.7 ± 1.3	0.084	17	26.0 ± 1.3	114.4	0.209	19	24.3 ± 1.3	107.0	0.786	19	21.8 ± 1.3	95.9	0.961	16	27.4 ± 1.3	120.5	**0.037**
104	17	24.2 ± 1.1	0.058	16	25.5 ± 1.1	105.6	0.799	19	24.3 ± 1.1	100.5	1.000	17	23.9 ± 1.1	99.1	1.000	15	27.5 ± 1.1	113.9	0.110
Mean for Weeks																			
4 - 104		19.5 ± 0.4			20.7 ± 0.4				19.6 ± 0.4				18.9 ± 0.4				20.3 ± 0.4		

[a] Week indicates the last week of a 4-week interval of daily food consumption, measured weekly by cage.
[b] N = Number of cages.
[c] Mean ± SE (g per day) = Estimated least squares mean and standard error.
[d] P-values in the 0.0 mM acrylamide column are the p-values for the trend test; p-values in the treatment columns are Dunnett's adjusted p-values for pairwise comparisons of the dose groups to the 0.0 mM acrylamide group.
[e] Pct = Ratio of the mean food consumption of the dose groups to the mean food consumption of the 0.0 mM acrylamide group, expressed as a percent.

TABLE G2
Food Consumption of Female Rats in the 2-Year Drinking Water Study of Acrylamide

Week[a]	0.0 mM			0.0875 mM				0.175 mM				0.35 mM				0.70 mM			
	N[b]	Mean ± SE[c]	P-Value[d]	N	Mean ± SE	Pct[e]	P-Value	N	Mean ± SE	Pct	P-Value	N	Mean ± SE	Pct	P-Value	N	Mean ± SE	Pct	P-Value
4	24	11.9 ± 0.1	0.548	24	12.0 ± 0.1	100.5	0.991	24	12.0 ± 0.1	100.7	0.964	24	11.8 ± 0.1	99.1	0.921	24	11.9 ± 0.1	99.7	0.999
8	24	11.7 ± 0.1	**0.008**	24	12.1 ± 0.1	102.8	0.328	24	11.9 ± 0.1	101.5	0.817	24	11.6 ± 0.1	98.8	0.894	24	11.4 ± 0.1	97.4	0.398
12	24	11.6 ± 0.1	**<0.001**	24	11.6 ± 0.1	100.4	0.997	24	11.5 ± 0.1	99.8	1.000	24	11.3 ± 0.1	97.5	0.337	24	10.9 ± 0.1	94.5	**0.003**
16	24	11.3 ± 0.1	**0.005**	24	11.6 ± 0.1	102.5	0.475	24	11.4 ± 0.1	101.1	0.937	24	11.3 ± 0.1	100.0	1.000	24	10.9 ± 0.1	96.5	0.187
20	24	11.4 ± 0.1	**0.003**	24	11.7 ± 0.2	103.3	0.236	24	11.5 ± 0.1	101.4	0.863	24	11.3 ± 0.1	99.8	1.000	24	11.0 ± 0.2	96.7	0.219
24	24	11.8 ± 0.2	**<0.001**	24	11.8 ± 0.2	99.8	1.000	24	11.5 ± 0.2	97.4	0.465	24	11.5 ± 0.2	96.7	0.244	24	11.1 ± 0.2	94.0	**0.007**
28	24	11.7 ± 0.1	**0.026**	24	11.9 ± 0.1	101.4	0.839	24	11.6 ± 0.1	98.9	0.924	24	11.7 ± 0.1	99.5	0.996	24	11.4 ± 0.1	96.9	0.244
32	24	11.8 ± 0.1	**0.025**	24	11.7 ± 0.1	99.7	0.999	24	11.7 ± 0.1	99.2	0.956	24	11.6 ± 0.1	98.6	0.778	24	11.4 ± 0.1	96.9	0.137
36	24	11.9 ± 0.1	**0.007**	24	11.7 ± 0.1	98.4	0.689	24	11.7 ± 0.1	98.5	0.738	24	11.7 ± 0.1	98.2	0.623	24	11.4 ± 0.1	95.7	**0.020**
40	24	12.1 ± 0.2	0.057	24	12.3 ± 0.2	101.8	0.845	24	12.0 ± 0.2	99.5	0.999	24	11.9 ± 0.2	98.4	0.881	24	11.7 ± 0.2	97.1	0.509
44	24	12.6 ± 0.2	0.129	24	12.2 ± 0.2	96.9	0.445	24	12.2 ± 0.2	96.5	0.330	24	12.4 ± 0.2	98.2	0.848	24	12.0 ± 0.2	95.3	0.124
48	24	13.1 ± 0.2	**0.017**	24	13.1 ± 0.2	100.0	1.000	24	12.9 ± 0.2	98.9	0.977	24	13.0 ± 0.2	99.4	0.998	24	12.4 ± 0.2	94.5	0.098
52	24	14.0 ± 0.3	0.643	24	14.0 ± 0.3	100.4	1.000	24	14.0 ± 0.3	100.1	1.000	24	14.1 ± 0.3	101.2	0.982	24	13.8 ± 0.3	98.8	0.979
56	24	15.0 ± 0.3	0.226	24	14.8 ± 0.3	98.7	0.973	24	14.6 ± 0.3	97.6	0.791	24	15.1 ± 0.3	100.6	0.999	24	15.3 ± 0.3	101.9	0.902
60	24	15.5 ± 0.3	0.371	24	15.2 ± 0.3	98.0	0.897	24	15.2 ± 0.3	98.1	0.912	24	15.2 ± 0.3	97.9	0.874	24	15.0 ± 0.3	97.0	0.668
64	24	15.3 ± 0.4	0.853	24	15.3 ± 0.4	100.2	1.000	24	15.6 ± 0.4	102.0	0.943	24	15.5 ± 0.4	101.5	0.983	23	15.3 ± 0.4	99.6	1.000
68	24	15.8 ± 0.4	0.576	24	15.9 ± 0.4	100.6	0.999	24	15.9 ± 0.4	101.1	0.992	24	16.2 ± 0.4	103.0	0.764	22	15.5 ± 0.4	98.2	0.952
72	24	16.0 ± 0.3	0.503	24	16.0 ± 0.3	100.1	1.000	24	16.4 ± 0.3	102.7	0.782	24	16.8 ± 0.3	105.3	0.254	22	16.2 ± 0.4	101.4	0.975
76	24	16.0 ± 0.4	0.077	24	15.5 ± 0.4	96.9	0.831	24	16.1 ± 0.4	101.2	0.994	24	17.1 ± 0.4	107.0	0.197	22	16.5 ± 0.4	103.7	0.738
80	24	16.6 ± 0.5	0.890	23	16.6 ± 0.5	99.7	1.000	24	16.4 ± 0.5	98.8	0.997	24	17.7 ± 0.5	106.1	0.435	22	16.5 ± 0.5	99.0	0.998
84	24	17.2 ± 1.1	0.094	23	16.5 ± 1.1	96.4	0.983	24	17.3 ± 1.1	100.7	1.000	24	18.2 ± 1.1	106.0	0.903	21	19.1 ± 1.1	111.1	0.548
88	23	17.2 ± 0.6	0.058	23	16.9 ± 0.6	98.6	0.996	23	18.9 ± 0.6	110.4	0.119	24	19.3 ± 0.6	112.2	**0.048**	20	18.5 ± 0.6	107.8	0.354
92	23	16.9 ± 0.8	**0.037**	23	17.5 ± 0.8	103.5	0.952	23	19.4 ± 0.8	114.6	0.073	24	19.7 ± 0.8	116.3	**0.035**	19	19.2 ± 0.8	113.3	0.132
96	23	17.0 ± 0.7	0.133	23	18.1 ± 0.7	106.3	0.671	21	19.1 ± 0.7	112.0	0.144	24	19.2 ± 0.7	112.5	0.112	18	18.9 ± 0.8	110.8	0.239
100	23	17.5 ± 1.0	0.234	23	20.0 ± 1.0	114.4	0.197	20	19.3 ± 1.0	110.4	0.483	23	20.0 ± 1.0	114.3	0.197	17	19.9 ± 1.0	113.7	0.266
104	22	17.9 ± 0.9	**0.048**	23	18.7 ± 0.9	104.6	0.912	19	19.2 ± 0.9	107.3	0.686	22	18.8 ± 0.9	105.4	0.854	16	20.7 ± 1.0	116.0	0.097
Mean for Weeks																			
4 – 104		14.3 ± 0.3			14.4 ± 0.3				14.6 ± 0.3				14.8 ± 0.3				14.5 ± 0.3		

[a] Week indicates the last week of a 4-week interval of daily food consumption, measured weekly by cage.
[b] N = Number of cages.
[c] Mean ± SE (g per day) = Estimated least squares mean and standard error.
[d] P-values in the 0.0 mM acrylamide column are the p-values for the trend test; p-values in the treatment columns are Dunnett's adjusted p-values for pairwise comparisons of the dose groups to the 0.0 mM acrylamide group.
[e] Pct = Ratio of the mean food consumption of the dose groups to the mean food consumption of the 0.0 mM acrylamide group, expressed as a percent.

TABLE G3
Food Consumption of Male Mice in the 2-Year Drinking Water Study of Acrylamide

Week[a]	0.0 mM			0.0875 mM				0.175 mM				0.35 mM				0.70 mM			
	N[b]	Mean ± SE[c]	P-Value[d]	N	Mean ± SE	Pct[e]	P-Value	N	Mean ± SE	Pct	P-Value	N	Mean ± SE	Pct	P-Value	N	Mean ± SE	Pct	P-Value
4	12	3.1 ± 0.2	0.223	12	3.1 ± 0.2	99.9	1.000	12	3.2 ± 0.2	104.6	0.973	12	3.0 ± 0.2	98.4	1.000	12	3.5 ± 0.2	111.9	0.563
8	12	3.1 ± 0.3	0.060	12	3.1 ± 0.3	98.8	1.000	12	3.4 ± 0.3	106.6	0.954	12	3.1 ± 0.3	99.6	1.000	13	3.8 ± 0.3	120.7	0.239
12	12	3.4 ± 0.3	**0.024**	12	3.4 ± 0.3	98.4	1.000	12	3.5 ± 0.3	100.8	1.000	12	3.4 ± 0.3	99.4	1.000	13	4.3 ± 0.3	124.6	0.154
16	12	3.9 ± 0.5	0.086	12	4.4 ± 0.5	113.2	0.843	12	4.3 ± 0.5	110.3	0.925	12	4.3 ± 0.5	110.4	0.924	13	5.0 ± 0.4	128.9	0.197
20	12	5.2 ± 0.7	0.772	12	5.0 ± 0.7	97.2	1.000	12	5.2 ± 0.7	100.2	1.000	12	5.2 ± 0.7	100.3	1.000	13	5.4 ± 0.6	103.6	0.999
24	12	3.9 ± 0.3	0.220	12	3.8 ± 0.3	97.0	0.997	12	3.8 ± 0.3	96.6	0.996	12	3.8 ± 0.3	96.8	0.997	13	4.4 ± 0.3	111.4	0.723
28	12	3.9 ± 0.3	**0.033**	12	3.8 ± 0.3	98.3	1.000	12	3.9 ± 0.3	98.8	1.000	12	3.8 ± 0.3	97.9	0.999	13	4.7 ± 0.3	120.6	0.199
32	12	4.0 ± 0.3	0.094	12	3.9 ± 0.3	98.2	0.999	12	3.9 ± 0.3	97.9	0.999	12	3.9 ± 0.3	98.5	1.000	13	4.5 ± 0.3	113.6	0.430
36	12	3.9 ± 0.4	0.147	12	3.8 ± 0.4	96.9	0.998	12	3.8 ± 0.4	98.9	1.000	12	3.7 ± 0.4	95.5	0.991	13	4.5 ± 0.3	115.5	0.541
40	12	3.8 ± 0.3	0.059	12	3.7 ± 0.3	96.4	0.993	12	3.8 ± 0.3	100.5	1.000	12	3.6 ± 0.3	95.4	0.982	13	4.5 ± 0.3	118.0	0.289
44	12	3.6 ± 0.3	**0.020**	12	3.6 ± 0.3	100.8	1.000	12	3.7 ± 0.3	102.5	0.999	12	3.6 ± 0.3	100.6	1.000	13	4.6 ± 0.3	128.3	0.110
48	12	3.7 ± 0.4	0.087	12	3.6 ± 0.4	98.3	1.000	12	3.6 ± 0.4	98.2	1.000	12	3.6 ± 0.4	98.6	1.000	13	4.4 ± 0.3	119.3	0.391
52	12	3.8 ± 0.3	**0.035**	12	3.7 ± 0.3	97.1	0.998	12	3.8 ± 0.3	99.3	1.000	12	3.6 ± 0.3	94.4	0.974	13	4.7 ± 0.3	122.1	0.193
56	12	3.7 ± 0.3	0.063	12	3.4 ± 0.3	90.6	0.743	12	3.5 ± 0.3	95.0	0.962	12	3.5 ± 0.3	93.9	0.928	13	4.2 ± 0.2	112.5	0.503
60	12	3.6 ± 0.3	**0.048**	12	3.4 ± 0.3	94.7	0.974	12	3.5 ± 0.3	97.5	0.999	12	3.4 ± 0.3	94.3	0.966	13	4.3 ± 0.3	118.2	0.298
64	12	3.6 ± 0.4	0.326	12	3.4 ± 0.4	92.4	0.963	12	3.4 ± 0.4	94.6	0.989	12	3.4 ± 0.4	93.7	0.980	13	4.0 ± 0.4	109.6	0.906
68	12	3.6 ± 0.3	0.099	12	3.4 ± 0.3	94.9	0.984	12	3.8 ± 0.3	105.7	0.976	12	3.4 ± 0.3	95.9	0.993	13	4.2 ± 0.3	118.2	0.392
72	12	3.8 ± 0.4	0.277	12	3.6 ± 0.4	94.5	0.983	12	3.9 ± 0.4	102.3	0.999	12	3.5 ± 0.4	91.4	0.921	13	4.3 ± 0.3	112.6	0.737
76	12	3.8 ± 0.3	0.062	12	3.7 ± 0.3	96.7	0.994	12	3.7 ± 0.3	97.1	0.996	12	3.7 ± 0.3	97.5	0.998	13	4.4 ± 0.3	115.8	0.352
80	12	3.9 ± 0.3	**0.027**	12	3.6 ± 0.3	92.7	0.926	12	3.9 ± 0.3	101.4	1.000	12	3.7 ± 0.3	94.1	0.964	13	4.7 ± 0.3	120.8	0.201
84	12	3.7 ± 0.3	**0.025**	12	3.4 ± 0.3	90.8	0.758	12	3.6 ± 0.3	97.4	0.997	12	3.5 ± 0.3	94.6	0.953	13	4.3 ± 0.2	116.1	0.276
88	12	3.7 ± 0.3	**<0.001**	12	3.5 ± 0.3	94.0	0.964	12	3.5 ± 0.3	94.1	0.965	12	3.6 ± 0.3	95.3	0.985	13	5.0 ± 0.3	134.9	**0.009**
92	12	4.2 ± 0.4	0.176	12	4.6 ± 0.4	110.2	0.886	12	4.4 ± 0.4	104.4	0.994	12	4.3 ± 0.4	102.3	1.000	13	5.1 ± 0.4	121.0	0.353
96	12	4.3 ± 0.4	**0.005**	12	4.2 ± 0.4	97.3	0.999	12	4.4 ± 0.4	101.8	1.000	12	4.1 ± 0.4	95.6	0.994	13	5.8 ± 0.4	135.2	**0.042**
100	12	4.3 ± 0.5	**0.025**	12	4.7 ± 0.5	109.0	0.960	12	4.8 ± 0.5	112.2	0.890	12	4.4 ± 0.5	101.7	1.000	13	6.0 ± 0.5	139.2	0.066
104	12	4.4 ± 0.6	**0.017**	12	4.8 ± 0.6	109.7	0.965	12	4.8 ± 0.6	108.4	0.979	12	4.4 ± 0.6	99.6	1.000	13	6.4 ± 0.6	145.8	0.052
Mean for Weeks																			
4 - 104		3.8 ± 0.2			3.8 ± 0.2				3.9 ± 0.2				3.8 ± 0.2				4.6 ± 0.2		

[a] Week indicates the last week of a 4-week interval of daily food consumption, measured weekly by cage.
[b] N = Number of cages.
[c] Mean ± SE (g per day) = Estimated least squares mean and standard error.
[d] P-values in the 0.0 mM acrylamide column are the p-values for the trend test; p-values in the treatment columns are Dunnett's adjusted p-values for pairwise comparisons of the dose groups to the 0.0 mM acrylamide group.
[e] Pct = Ratio of the mean food consumption of the dose groups to the mean food consumption of the 0.0 mM acrylamide group, expressed as a percent.

TABLE G4
Food Consumption of Female Mice in the 2-Year Drinking Water Study of Acrylamide

Week[a]	0.0 mM			0.0875 mM				0.175 mM				0.35 mM				0.70 mM			
	N[b]	Mean ± SE[c]	P-Value[d]	N	Mean ± SE	Pct[e]	P-Value	N	Mean ± SE	Pct	P-Value	N	Mean ± SE	Pct	P-Value	N	Mean ± SE	Pct	P-Value
4	12	2.9 ± 0.1	0.591	12	2.9 ± 0.1	99.0	0.996	12	2.8 ± 0.1	94.6	0.390	12	2.8 ± 0.1	96.1	0.679	12	2.9 ± 0.1	97.5	0.903
8	12	3.7 ± 0.3	0.080	12	3.1 ± 0.3	85.5	0.435	12	2.9 ± 0.3	79.4	0.153	12	2.8 ± 0.3	76.7	0.085	12	2.9 ± 0.3	78.4	0.123
12	12	3.7 ± 0.3	0.280	12	3.4 ± 0.3	91.3	0.830	12	3.3 ± 0.3	88.6	0.660	12	3.4 ± 0.3	91.1	0.820	12	3.2 ± 0.3	85.8	0.469
16	12	4.0 ± 0.4	0.898	12	4.1 ± 0.4	104.3	0.995	12	4.3 ± 0.4	107.1	0.969	12	4.1 ± 0.4	103.1	0.999	12	4.0 ± 0.4	100.8	1.000
20	12	4.9 ± 0.7	0.822	12	5.0 ± 0.7	101.7	1.000	12	4.9 ± 0.7	98.2	1.000	12	4.9 ± 0.7	98.4	1.000	12	4.8 ± 0.7	96.6	0.999
24	12	3.7 ± 0.3	0.843	12	3.8 ± 0.3	101.0	1.000	12	4.2 ± 0.3	112.0	0.611	12	3.6 ± 0.3	95.2	0.974	12	3.8 ± 0.3	101.9	0.999
28	12	3.7 ± 0.1	0.294	12	3.9 ± 0.1	105.1	0.546	12	3.8 ± 0.1	102.2	0.959	12	3.6 ± 0.1	97.7	0.951	12	3.7 ± 0.1	99.0	0.998
32	12	3.9 ± 0.1	0.883	12	3.9 ± 0.1	101.2	0.991	12	3.8 ± 0.1	98.9	0.992	12	3.9 ± 0.1	102.0	0.947	12	3.8 ± 0.1	99.4	0.999
36	12	3.7 ± 0.1	0.862	12	3.9 ± 0.1	104.3	0.526	12	3.6 ± 0.1	96.3	0.650	12	3.8 ± 0.1	101.1	0.994	12	3.7 ± 0.1	100.2	1.000
40	12	3.6 ± 0.1	0.642	12	3.7 ± 0.1	102.6	0.894	12	3.7 ± 0.1	102.3	0.931	12	3.7 ± 0.1	102.8	0.862	12	3.6 ± 0.1	99.4	0.999
44	12	3.6 ± 0.1	0.772	12	3.7 ± 0.1	102.7	0.945	12	3.8 ± 0.1	106.4	0.455	12	3.7 ± 0.1	102.2	0.973	12	3.6 ± 0.1	100.7	1.000
48	12	3.6 ± 0.1	0.651	12	3.7 ± 0.1	100.5	1.000	12	3.7 ± 0.1	102.2	0.953	12	3.7 ± 0.1	101.9	0.971	12	3.7 ± 0.1	101.9	0.972
52	12	3.7 ± 0.2	0.870	12	3.9 ± 0.2	105.9	0.713	12	3.9 ± 0.2	106.5	0.633	12	3.9 ± 0.2	105.3	0.783	12	3.8 ± 0.2	103.5	0.939
56	12	3.7 ± 0.1	0.546	12	3.7 ± 0.1	100.6	1.000	12	3.6 ± 0.1	97.5	0.972	12	3.7 ± 0.1	101.6	0.995	12	3.5 ± 0.1	96.5	0.914
60	12	3.6 ± 0.1	0.987	12	3.7 ± 0.1	103.7	0.889	12	3.7 ± 0.1	102.5	0.968	12	3.7 ± 0.1	103.7	0.890	12	3.6 ± 0.1	101.2	0.998
64	12	3.4 ± 0.1	0.439	12	3.4 ± 0.1	101.3	0.994	12	3.4 ± 0.1	99.2	0.999	12	3.4 ± 0.1	100.4	1.000	12	3.5 ± 0.1	103.2	0.865
68	12	3.6 ± 0.1	0.155	12	3.6 ± 0.1	99.7	1.000	12	3.5 ± 0.1	98.1	0.989	12	3.7 ± 0.1	104.2	0.840	12	3.8 ± 0.1	105.3	0.702
72	12	3.7 ± 0.1	0.277	12	3.7 ± 0.1	100.0	1.000	12	3.7 ± 0.1	100.4	1.000	12	3.8 ± 0.1	103.3	0.938	12	3.8 ± 0.1	104.9	0.798
76	12	3.7 ± 0.1	0.195	12	3.8 ± 0.1	103.1	0.919	12	3.9 ± 0.1	105.0	0.691	12	4.1 ± 0.1	110.5	0.098	12	3.9 ± 0.1	105.7	0.577
80	12	3.8 ± 0.2	0.064	12	3.6 ± 0.2	95.5	0.885	12	4.1 ± 0.2	107.9	0.520	12	4.4 ± 0.2	115.2	0.053	12	4.1 ± 0.2	107.1	0.613
84	12	3.8 ± 0.2	**0.028**	12	3.6 ± 0.2	95.4	0.933	12	3.8 ± 0.2	99.0	1.000	12	4.3 ± 0.2	113.1	0.250	12	4.2 ± 0.2	110.3	0.459
88	12	3.9 ± 0.3	**0.003**	12	3.6 ± 0.3	93.0	0.905	12	3.9 ± 0.3	99.1	1.000	12	4.6 ± 0.3	117.0	0.294	12	4.7 ± 0.3	121.2	0.135
92	12	3.9 ± 0.5	**<0.001**	12	4.5 ± 0.5	116.8	0.779	12	4.2 ± 0.5	108.9	0.970	12	6.0 ± 0.5	153.4	**0.015**	12	6.3 ± 0.5	162.5	**0.003**
96	12	4.3 ± 0.5	**0.001**	12	4.4 ± 0.5	102.9	0.999	12	4.5 ± 0.5	105.2	0.993	12	5.7 ± 0.5	133.9	0.113	12	6.2 ± 0.5	144.1	**0.022**
100	12	4.3 ± 0.5	**<0.001**	12	5.3 ± 0.5	121.3	0.503	12	4.7 ± 0.5	107.9	0.970	12	5.8 ± 0.5	134.7	0.114	12	7.3 ± 0.5	167.8	**<0.001**
104	12	4.6 ± 0.5	**<0.001**	12	5.3 ± 0.5	114.6	0.780	12	4.9 ± 0.5	104.7	0.995	12	6.8 ± 0.5	146.4	**0.016**	11	8.1 ± 0.5	173.8	**<0.001**
Mean for Weeks																			
4 - 104		3.8 ± 0.1			3.9 ± 0.1				3.9 ± 0.1				4.1 ± 0.1				4.3 ± 0.1		

[a] Week indicates the last week of a 4-week interval of daily food consumption, measured weekly by cage.
[b] N = Number of cages.
[c] Mean ± SE (g per day) = Estimated least squares mean and standard error.
[d] P-values in the 0.0 mM acrylamide column are the p-values for the trend test; p-values in the treatment columns are Dunnett's adjusted p-values for pairwise comparisons of the dose groups to the 0.0 mM acrylamide group.
[e] Pct = Ratio of the mean food consumption of the dose groups to the mean food consumption of the 0.0 mM acrylamide group, expressed as a percent.

APPENDIX H
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH-31 IR RAT AND MOUSE RATION

TABLE H1
Ingredients of NIH-31 IR Rat and Mouse Ration

Ingredients[a]	Percent by Weight
Ground whole hard wheat	35.5
Ground #2 yellow shelled corn	21.0
Ground whole oats	10.0
Wheat middlings	10.0
Fish meal (60% protein)	9.0
Soybean meal (48.5% protein)	5.0
Alfalfa meal (17% protein)	2.0
Corn gluten meal (60%)	2.0
Dicalcium phosphate[b]	1.5
Soy oil	1.5
Brewers dried yeast	1.0
Ground limestone[b]	0.5
Premixes	0.5
Salt	0.5

[a] Ingredients ground to pass through a U.S. Standard Screen No. 16 before mixing.
[b] Specific ingredient requirement for cadmium content not to exceed 1 mg/kg.

TABLE H2
Vitamins and Minerals in NIH-31 IR Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	22,000,000 IU	Vitamin A palmitate or acetate
D_3	3,800,000 IU	D-activated animal sterol
K_3	20 g	Menadione activity
Choline	700 g	Choline chloride
dl-α-Tocopheryl acetate	15 g	
Folic acid	1 g	
Niacin	20 g	
d-Pantothenic acid	25 g	*d*-Calcium pantothenate
Riboflavin	5 g	
Thiamine	65 g	Thiamine mononitrate
B_{12}	14 g	
Pyridoxine	2 g	Pyridoxine hydrochloride
Biotin	0.12 g	*d*-Biotin
Minerals		
Magnesium	400 g	Magnesium oxide
Manganese	100 g	Manganese oxide
Iron	60 g	Iron sulfate
Zinc	10 g	Zinc oxide
Copper	4 g	Copper sulfate
Iodine	1.5 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

[a] Per ton (2,000 lb) of finished product.

TABLE H3
Nutrient Composition of NIH-31 IR Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Number of Samples
Crude protein (% by weight)	18.3 ± 0.5	10
Crude fat (% by weight)	5.24 ± 0.60	10
Volatiles (% by weight)	8.50 ± 0.49	10
Vitamin		
A (μg/g)	3.09 ± 0.57	10
E (μg/g)	38.5 ± 11.9	10
B1 (μg/g)	26.0 ± 3.4	10
Mineral		
Selenium (μg/g)	0.39 ± 0.08	10

TABLE H4
Contaminant Levels in NIH-31 IR Rat and Mouse Ration

	Mean ± Standard Deviation	Number of Samples (Number Positive)
Contaminants		
Acrylamide (ppb)	28.1 ± 24.7	10 (9)
Arsenic (μg/g)	0.18 ± 0.02	10 (10)
Cadmium (μg/g)	0.19 ±0.07	10 (10)
Lead (μg/g)	0.42 ±0.09	10 (10)
Aflatoxin B1 (ppb)	<mdl	10 (0)
Aflatoxin B2 (ppb)	<mdl	10 (0)
Aflatoxin G1 (ppb)	<mdl	10 (0)
Aflatoxin G2 (ppb)	<mdl	10 (0)
Total Fumonisin (ppb)	343 ± 213	10 (10)
Pesticides (ppb)		
Heptachlor	<mdl	1 (0)
Total DDT	<mdl	1 (0)
Dieldrin	<mdl	1 (0)
PCB	<mdl	1 (0)
Malathion	<mdl	1 (0)
Lindane	<mdl	1 (0)

APPENDIX I
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

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

Blood from each sentinel animal was collected, allowed to clot and the serum was separated. The serum was analyzed by Multiplex Fluorescent Immunoassay (MFI) for the presence of specific antibodies by the Research Animal Diagnostic Laboratory, University of Missouri, Columbia, Missouri. The laboratory serology method and viral/mycoplasma agent 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
MICE	
MFI	
Mouse Hepatitis Virus (MHV)	26, 53, 79, 104, and 117 weeks
Sedai	26, 53, 79, 104, and 117 weeks
Pneumonia Virus of Mice (PVM)	26, 53, 79, 104, and 117 weeks
Reovirus Type 3 (REO3)	26, 53, 79, 104, and 117 weeks
Theiler's Murine Encephalomyelitis Virus (TMEV)	26, 53, 79, 104, and 117 weeks
Ectromelia	26, 53, 79, 104, and 117 weeks
Polyoma	26, 53, 79, 104, and 117 weeks
Mycoplasma pulmonis	26, 53, 79, 104, and 117 weeks
Minute Virus of Mice (MMV)	26, 53, 79, 104, and 117 weeks
Mouse Parvovirus (MPV)	26, 53, 79, 104, and 117 weeks
Parvo NS-1	26, 53, 79, 104, and 117 weeks
Epizootic Diarrhea of Infant Mice Virus (EDIM)	26, 53, 79, 104, and 117 weeks
Lymphocytic Choriomeningitis Virus (LCM)	26, 53, 79, 104, and 117 weeks
RATS	
MFI	
Rat Coronavirus/Sialodacryoadenitis (RCV/SDAV)	26, 53, 79, and 104 weeks
Sendai	26, 53, 79, and 104 weeks
Pneumonia Virus of Mice (PVM)	26, 53, 79, and 104 weeks
TMEV GDVII	26, 53, 79, and 104 weeks
Mycoplasma pulmonis	26, 53, 79, and 104 weeks
Parvo NS-1	26, 53, 79, and 104 weeks

RATS AND MICE

Additional Screening

Bordetella bronchiseptica	*Listeria monocytogenes*	Ectoparasites
Citrobacter freundii	*Pasteurella pneumontropica*	Endoparasites
Corynebacterium kutscheri	*Pasteurella multocida*	
Erysipelothrix rhusiopathiae	*Pseudomonas aeruginosa*	
Helicobacter bilis	*Salmonella* sp.	
Helicobacter hepaticus	*Streptococcus pneumoniae*	

RESULTS

All serology test results were negative.

Helicobacter hepaticus was detected via polymerase chain reaction (PCR) in three of the sentinel mice. *Pseudomonas aeruginosa* was detected in two of the sentinel rats.