NTP
National Toxicology Program
U.S. Department of Health and Human Services

NTP Genetically Modified Model Report on the

Toxicology Studies of Aspartame (CASRN 22839-47-0) in Genetically Modified (FVB Tg.AC Hemizygous) and B6.129-Cdkn2a^{tm1Rdp} (N2) Deficient Mice and Carcinogenicity Studies of Aspartame in Genetically Modified [B6.129-*Trp53*^{tm1Brd} (N5) Haploinsufficient] Mice (Feed Studies)

NTP GMM 01

OCTOBER 2005

NTP REPORT

ON THE

TOXICOLOGY STUDIES OF ASPARTAME
(CAS NO. 22839-47-0)

IN GENETICALLY MODIFIED (FVB Tg.AC HEMIZYGOUS) AND B6.129-Cdkn2a^{tm1Rdp} (N2) DEFICIENT MICE

AND CARCINOGENICITY STUDIES OF ASPARTAME

IN GENETICALLY MODIFIED [B6.129-*Trp53*^{tm1Brd} (N5) HAPLOINSUFFICIENT] MICE

(FEED STUDIES)

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

October 2005

NTP GMM 1

NIH Publication No. 06-4459

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

FOREWORD

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

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

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

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

Details about ongoing and completed NTP studies, abstracts of all NTP Technical Reports, and full versions of the completed reports are available at the NTP's World Wide Web site: http://ntp.niehs.nih.gov. In addition, printed copies of these reports are available from NTP as supplies last by contacting (919) 541-3419.

CONTRIBUTORS

National Toxicology Program

Evaluated and interpreted results and reported findings

J.R. Bucher, Ph.D., Study Scientist
D.W. Bristol, Ph.D.
J.E. French, Ph.D.
J.R. Hailey, D.V.M.
J.K. Haseman, Ph.D.
R.A. Herbert, D.V.M., Ph.D.
D.E. Malarkey, D.V.M., Ph.D.
R.R. Maronpot, D.V.M.
J.C. Peckham, D.V.M., M.S., Ph.D.
J.H. Roycroft, Ph.D.
C.S. Smith, Ph.D.
G.S. Travlos, D.V.M.
M.K. Vallant, B.S., M.T.
K.L. Witt, M.S., ILS, Inc.

BioReliance Corporation

Conducted studies and evaluated pathology findings

M.L. Wenk, Ph.D., Principal Investigator
L.L. Lanning, D.V.M.

Experimental Pathology Laboratories, Inc.

Provided pathology review

J.F. Hardisty, D.V.M., Principal Investigator
C.C. Shackelford, D.V.M., M.S., Ph.D.

Dynamac Corporation

Prepared quality assurance audits

S. Brecher, Ph.D., Principal Investigator

NTP Pathology Working Group

Evaluated slides and prepared pathology report (April 15, 2002)

P. Long, D.V.M., Ph.D., Chairperson
Pathology Associates International
D. Dixon, D.V.M., Ph.D.
National Toxicology Program
G.P. Flake, M.D.
National Toxicology Program
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
P.B. Little, D.V.M., M.S., Ph.D.
Pathology Associates International
A. Nyska, D.V.M.
National Toxicology Program
C.C. Shackelford, D.V.M., M.S., Ph.D.
Experimental Pathology Laboratories, Inc.

Analytical Sciences, Inc.

Provided statistical analyses

P.W. Crockett, Ph.D., Principal Investigator
L.J. Betz, M.S.
K.P. McGowan, M.B.A.
J.T. Scott, M.S.

Biotechnical Services, Inc.

Prepared Report

S.R. Gunnels, M.A., Principal Investigator
P.H. Carver, B.A.
K.K. Coker, Ph.D.
B.F. Hall, M.S.
L.M. Harper, B.S.
E.S. Rathman, M.S.
D.C. Serbus, Ph.D.
R.A. Willis, B.A., B.S.

CONTENTS

SUMMARY

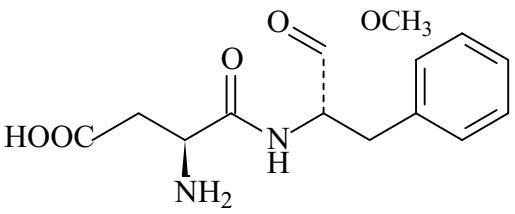
Background
Aspartame is an artificial sweetener widely used in beverages and foods. We tested if aspartame could cause cancer in two different strains of genetically modified mice.

Methods
We fed groups of male and female Tg.AC mice, male and female p53 mice, and male and female Cdkn2a mice diets containing up to 50,000 parts per million (5%) aspartame for 9 months. Animals given feed with no sweetener added served as the control groups. Tissues from 15 sites were examined for every animal.

Results
Exposure to aspartame had no effect on the survival of any of the animal groups. No increases in tumors were seen in males or females from either strain of mice.

Conclusions
We conclude that aspartame did not cause cancer in the genetically modified mice used in these studies.

ABSTRACT



ASPARTAME

CAS No. 22839-47-0

Chemical Formula: $C_{14}H_{18}N_2O_5$ Molecular Weight: 294.3

Synonyms: *N*-L-α-Aspartyl-L-phenylalanine 1-methyl ester; 3-amino-*N*-(α-carboxyphenethyl)succinamic acid *N*-methyl ester; APM; SC-18862
Trade names: Canderel, Equal, NutraSweet, Sanecta, Tri-Sweet

Aspartame is an artificial sweetener used throughout the world in food and beverages. Conventional 2-year rodent cancer studies of aspartame are considered negative, although a small number of neoplasms of the brain were observed in a rat study (*Fed. Regist.,* 1981a,b). The NTP has explored the use of genetically altered mouse models as adjuncts to the 2-year rodent cancer assay. These models may prove to be more rapid, use fewer animals, and provide some mechanistic insights into neoplastic responses. As part of the evaluation of new mouse cancer screening models, aspartame was tested for potential toxicity and carcinogenicity in two relatively well-studied models, the Tg.AC hemizygous strain and the p53 haploinsufficient strain, and an uncharacterized model, the Cdkn2a deficient strain. Male and female Tg.AC hemizygous, p53 haploinsufficient, and Cdkn2a deficient mice were given feed containing aspartame (greater than 98% pure) for 9 months. Genetic toxicology studies were conducted in *Salmonella typhimurium,* rat bone marrow cells, and mouse peripheral blood erythrocytes.

9-Month Study in Tg.AC Hemizygous Mice

Groups of 15 male and 15 female Tg.AC hemizygous mice were fed diets containing 0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm aspartame (equivalent to average daily doses of approximately 490, 980, 1,960, 3,960, or 7,660 mg aspartame/kg body weight to males and 550, 1,100, 2,260, 4,420, or 8,180 mg/kg to females) for 40 weeks. Exposure to aspartame had no effect on survival. The mean body weights of 50,000 ppm females were greater than those of the controls from week 15 until the end of the study. Feed consumption by the

exposed groups was similar to that by the control groups throughout the study. There were no neoplasms or nonneoplastic lesions that were attributed to exposure to aspartame.

9-Month Study in p53 Haploinsufficient Mice

Groups of 15 male and 15 female p53 haploinsufficient mice were fed diets containing 0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm aspartame (equivalent to average daily doses of approximately 490, 970, 1,860, 3,800, or 7,280 mg/kg to males and 630, 1,210, 2,490, 5,020, or 9,620 mg/kg to females) for 40 weeks. Exposure to aspartame had no effect on survival or mean body weights. Feed consumption by the exposed groups was similar to that by the control groups throughout the study. No neoplasms or nonneoplastic lesions were attributed to exposure to aspartame.

9-Month Study in Cdkn2a Deficient Mice

Groups of 15 male and 15 female Cdkn2a deficient mice were fed diets containing 0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm aspartame for 40 weeks (equivalent to average daily doses of approximately of approximately 490, 960, 1,900, 3,700, and 7,400 mg/kg to males and 610, 1,200, 2,390, 4,850, and 9,560 mg/kg to females). Survival of all exposed groups was similar to that of the control groups. Mean body weights of 3,125 and 6,250 ppm males were less than those of the controls after weeks 29 and 16, respectively. Mean body weights of female mice were similar to those of the controls throughout the study. The incidences of minimal to mild cytoplasmic vacuolization of periportal hepatocytes were significantly greater than controls in males exposed to 6,250, 25,000, or 50,000 ppm aspartame.

Genetic Toxicology

Aspartame was tested for induction of gene mutations in *Salmonella typhimurium.* No mutagenicity was detected in strains TA98, TA100, or TA1535 with or without exogenous metabolic activation (S9). In addition, a single test in TA1537 with 30% rat liver S9 gave negative results. In TA97 with 30% rat liver S9, however, a reproducible small increase in mutant colonies was observed, and this response was judged to be equivocal. No mutagenicity was detected in TA97 without S9 or with hamster liver S9.

An acute bone marrow micronucleus test was conducted with aspartame administered by gavage to male F344/N rats. No increase in micronucleated polychromatic erythrocytes was observed at any dose level.

Peripheral blood micronucleus tests were conducted after 9 months exposure of Tg.AC hemizygous, p53 haploinsufficient, and Cdkn2a deficient mice to aspartame in dosed feed. Negative results were obtained in male and female Tg.AC hemizygous and Cdkn2a deficient mice. Negative results were also obtained with male p53 haploinsufficient mice. In female p53 haploinsufficient mice, the results of the micronucleus test were judged to be positive, based on a significant trend test and a small but statistically significant increased frequency of micronucleated erythrocytes in the 50,000 ppm group.

Conclusions

Under the conditions of this 9-month feed study, there was *no evidence of carcinogenic activity** of aspartame in male or female p53 haploinsufficient mice exposed to 3,125, 6,250, 12,500, 25,000, or 50,000 ppm. Because this is a new model, there is uncertainty whether the study possessed sufficient sensitivity to detect a carcinogenic effect.

---

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Report Review Subcommittee comments and public discussion on this report appears on page 11.

Summary of the Feed and Genetic Toxicology Studies of Aspartame in Tg.AC Hemizygous Mice

	Male	Female
Concentrations in feed	0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm	0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm
Body weights	Exposed groups similar to the control group	50,000 ppm group greater than control group
Survival rates	9/15, 12/15, 8/15, 12/15, 11/15, 10/15	11/15, 10/15, 9/15, 9/15, 11/15, 8/15
Nonneoplastic effects	None	None
Neoplastic effects	None	None
Genetic toxicology		
Salmonella typhimurium gene mutations:	Equivocal in TA97 with S9; negative in TA98, TA100, TA1535, and TA1537	
Micronucleated erythrocytes		
Rat bone marrow *in vivo*:	Negative	
Mouse peripheral blood *in vivo*:	Negative in Tg.AC hemizygous males and females	

Summary of the Feed and Genetic Toxicology Studies of Aspartame in p53 Haploinsufficient Deficient Mice

	Male	Female
Concentrations in feed	0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm	0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm
Body weights	Exposed groups similar to the control group	Exposed groups similar to the control group
Survival rates	14/15, 15/15, 13/15, 15/15, 14/15, 14/15	14/15, 14/15, 14/15, 15/15, 15/15, 15/15
Nonneoplastic effects	None	None
Neoplastic effects	None	None
Level of evidence of carcinogenic activity	No evidence	No evidence
Genetic toxicology		
Micronucleated erythrocytes		
Mouse peripheral blood *in vivo*:	Positive in p53 haploinsufficient females and negative in p53 haploinsufficient males	

Summary of the Feed and Genetic Toxicology Studies of Aspartame in Cdkn2a Deficient Mice

	Male	Female
Concentrations in feed	0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm	0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm
Body weights	3,125 ppm and 6,250 ppm groups less than control group	Exposed groups similar to the control group
Survival rates	14/15, 14/15, 15/15, 14/15, 14/15, 15/15	13/15, 15/15, 13/15, 15/15, 15/15, 14/15
Nonneoplastic effects	Liver: hepatocyte, cytoplasmic periportal vacuolization (6/15, 11/15, 14/15, 8/14, 13/15, 13/15)	None
Neoplastic effects	None	None
Genetic toxicology		
Micronucleated erythrocytes		
Mouse peripheral blood *in vivo*:	Negative in Cdkn2a deficient males and females	

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

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

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

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

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Report on aspartame on May 22, 2003, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the 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.

Mary Anna Thrall, D.V.M., Chairperson
Department of Pathology
College of Veterinary Medicine and Biomedical Sciences
Colorado State University
Fort Collins, CO

Kim Boekelheide, M.D., Ph.D.
Division of Biology and Medicine
Department of Pathology and Laboratory Medicine
Brown University
Providence, RI

Hillary M. Carpenter, III, Ph.D.
Minnesota Department of Health
St. Paul, MN

Michael R. Elwell, D.V.M., Ph.D., Principal Reviewer
Pfizer Global Research and Development
Groton, CT

Walter W. Piegorsch, Ph.D., Principal Reviewer
Department of Statistics
University of South Carolina
Columbia, SC

Stephen M. Roberts, Ph.D.
Department of Physiological Sciences
College of Veterinary Medicine
University of Florida
Gainesville, FL

Richard D. Storer, M.P.H., Ph.D., Principal Reviewer
Department of Genetic and Cellular Toxicology
Merck Research Laboratories
West Point, PA

Mary Vore, Ph.D.
Graduate Center for Toxicology
University of Kentucky
Lexington, KY

Cheryl Lyn Walker, Ph.D.
M.D. Anderson Cancer Center
The University of Texas
Smithville, TX

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On May 22, 2003, the draft Report on the toxicology and carcinogenesis studies of aspartame received public review by the National Toxicology Program's Board of Scientific Counselor's Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.R. Bucher, NIEHS, introduced a new report series consisting of studies performed with genetically modified mice. The toxicology and carcinogenicity studies of aspartame are the first report in that series. Dr. J.E. French, NIEHS, presented an overview of the Tg.AC hemizygous, p53 haploinsufficient, and Cdkn2a deficient mouse models used in this study. Dr. Bucher proceeded to describe the uses of aspartame, the rationale for study nomination, the design of the transgenic mouse studies, the pathology evaluation, and the body weight, survival, and neoplasm incidence results. For the new report series, the traditional Levels of Evidence of Carcinogenic Activity categories would be used to frame conclusions. The proposed conclusions were: *no evidence of carcinogenic activity* of aspartame in male or female p53 haploinsufficient mice.

Dr. Elwell, the first principal reviewer, agreed that the studies did not show evidence of a carcinogenic effect. He also stated that the report described well the relevance and uncertainties of positive and negative findings in these models. He inquired why positive controls were not used in the p53 haploinsufficient mouse study and suggested clarification that the uncertain findings in the literature suggesting brain tumors from this compound were limited to one study.

Dr. Storer, the second principal reviewer, questioned the statistical criteria used to score the micronucleus test, noting that in virtually every study some positive response was reported. He agreed with the proposed conclusions.

Dr. Piegorsch, the third principal reviewer, inquired about design considerations such as the number of animals per group and the spacing of doses, particularly for studies with negative findings. He felt the proposed conclusion statement was appropriate.

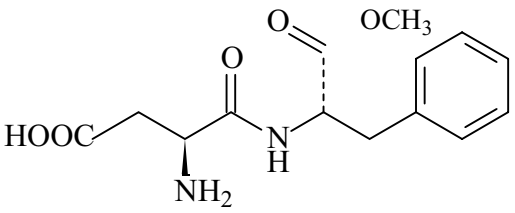
Dr. Bucher replied that for the p53 model, positive controls were not used because variations in response to positive control agents were viewed historically to be due to dosing irregularities rather than to a lack of responsiveness from the mouse model. He indicated that the procedure for scoring micronuclei was being changed from scoring slide smears to flow cytometry, which would enhance the power of the test. Dr. C.J. Portier, NIEHS, commented that one of the motivations for adopting the transgenic mouse models was to be able to use smaller numbers of animals, which would be justified for testing purposes if the background incidence rates for neoplasms prove to be low.

Dr. Elwell moved, and Dr. Storer seconded, that the conclusions be accepted as written. Dr. Roberts and Dr. Walker expressed concern that there was not sufficient historical information to determine whether the study design, particularly the number of animals per group, was adequate. Dr. French observed that in studies in these models, groups of 10 to 20 mice had been used, and considerations of study duration and potency of the chemical were also factors in eliciting positive responses. He suggested that in the absence of preneoplastic lesions, larger group sizes would not reveal any differences in response. Dr. Storer suggested there were two different questions of adequacy, one about the validity of the study protocol, and the other about the conduct and results of the study within that protocol. Deficiencies in the latter might fit the criteria for inadequate study, whereas questions about the overall validity of the study model might be addressed in other arenas, such as the Report on Carcinogens.

Dr. Carpenter suggested that some caveat be added to the conclusion reflecting reservations about the significance of a negative result in this type of study. Drs. Walker and Vore also endorsed adding some qualifying statement to the conclusions. The panel explored a variety of phrasings, and then agreed unanimously to add the following sentence: "Because this is a new model, there is uncertainty whether the study possessed sufficient sensitivity to detect a carcinogenic effect". The subcommittee then unanimously approved the original conclusion, with this sentence added.

There was subsequent discussion about adding results from the Tg.AC hemizygous and Cdkn2a deficient studies to the abstract table, to accompany the results from the p53 haploinsufficient model upon which the conclusions were based. Following discussion, it was agreed that results from the other models could be summarized in the abstract table but with no reference to levels of evidence of carcinogenic activity.

INTRODUCTION



ASPARTAME

CAS No. 22839-47-0

Chemical Formula: $C_{14}H_{18}N_2O_5$ Molecular Weight: 294.3

Synonyms: *N*-L-α-Aspartyl-L-phenylalanine 1-methyl ester; 3-amino-*N*-(α-carboxyphenethyl)succinamic acid *N*-methyl ester; APM; SC-18862
Trade names: Canderel, Equal, NutraSweet, Sanecta, Tri-Sweet

CHEMICAL AND PHYSICAL PROPERTIES

Aspartame (the methyl ester of the dipeptide L-aspartyl-L-phenylalanine) is an odorless off-white crystalline powder with a melting point of approximately 246.5° C. A 0.8% solution in water has a pH of 5.3. The solubility of aspartame in water at 25° C is approximately 18.2 mg/mL. Aspartame is more soluble in acidic solutions and hot water and is only slightly soluble in alcohol and chloroform. The dipeptide ester is about 160 times sweeter than sucrose in aqueous solutions. In the presence of moisture, aspartame will hydrolyze to form aspartylphenylalanine and a diketopiperazine derivative, resulting in the loss of sweetness (HSDB, 2003). Photodecomposition of aspartame may occur in aqueous solutions (Kim *et al.,* 1997), and aspartame may also decompose at high temperatures (Galletti *et al.,* 1995).

PRODUCTION, USE, AND HUMAN EXPOSURE

In 1974, the Food and Drug Administration (FDA) approved the use of aspartame as a sweetener in dry sugar substitutes in free-flowing and tablet form, cold cereals, chewing gum, and a dry base for beverages, instant coffees and teas, puddings and gelatins, and a dairy analogue topping (*Fed. Regist.,* 1981a,b). The FDA Commissioner issued a final decision on the safety of aspartame for use in these food products in 1981 (*Fed. Regist.,* 1981a,b). In 1983, the FDA also approved the use of aspartame as a sweetener in carbonated beverages and carbonated beverage syrup bases (*Fed. Regist.,* 1984). In 1996, the FDA amended the food additive regulations to provide for the safe use of aspartame as a general purpose sweetener; the acceptable daily intake is up to 50 mg aspartame/kg body weight per day (*Fed. Regist.,* 1996).

Aspartame is produced by the NutraSweet Company. In large-scale production of aspartame, L-aspartic acid anhydride is condensed with L-phenylalanine methyl ester to form a mixture of β-L-aspartyl-L-phenylalanine methyl ester and α-L-aspartyl-L-phenylalanine methyl ester. On acidification with hydrochloric acid, α-L-aspartyl-L-phenylalanine methyl ester precipitates, and the precipitate is neutralized to form aspartame (HSDB, 2003). It is estimated that 8,040 tons of aspartame are consumed in the United States each year (HSDB, 2003).

Aspartame is present in many food items such as beverages (180 mg/12 oz), yogurt (125 mg/8 oz), gelatin dessert (95 mg/4 oz), hot chocolate (50 mg/6 oz), tabletop sweetener (35 mg/1 packet), pudding desert (25 mg/4 oz), and breath mints (1.5 mg/mint), and the upper end (90th percentile) of the daily average intake of aspartame may be 3 to 5 mg/kg body weight (Butchko and Stargel, 2001).

ABSORPTION, DISTRIBUTION, AND EXCRETION

Studies in a number of animal species indicate that aspartame is rapidly and extensively metabolized to its constituent amino acids and methanol (Figure 1). The hydrolysis occurs during the absorption process (Ranney *et al.,* 1976; Ranney and Oppermann, 1979; Stegink and Filer, 1984; Stegink, 1987; Diomede *et al.,* 1991) (Figure 2). Aspartame (*N*-L-α-aspartyl-L-phenylalanine 1-methyl ester) is the methyl ester of a dipeptide, aspartylphenylalanine. The ester bond is rapidly hydrolyzed in the gastrointestinal tract in mice, rats, rabbits, dogs, monkeys, and humans to give methanol, aspartic acid, and phenylalanine (Ranney *et al.,* 1976). Methanol may be oxidized to formaldehyde and formic acid in the body (Trocho *et al.,* 1998).

When aspartame is administered with carbohydrate, which promotes insulin secretion, plasma levels of other large neutral amino acids that compete with phenylalanine and tyrosine for brain uptake are lowered, and the brain levels of phenylalanine may increase (Dews, 1987). In the fetus and in young children, very high plasma levels of phenylalanine may interfere with the development of the nervous system. This condition is also seen in phenylketonuria because of a lower level of phenylalanine hydroxylase activity (Dews, 1987).

Complete absorption and toxicokinetic data are not available to compare the disposition of aspartame in humans and animals over the same dose range. However, the available data suggest that animals clear aspartame and/or metabolites more rapidly than humans (Reynolds *et al.,* 1980).

Phenylalanine in plasma has been used as a marker for aspartame oral absorption in some studies. In humans, a normal plasma level of phenylalanine is 60 µM. Uncontrolled phenylketonurics have phenylalanine plasma levels of up to 1,200 µM. In humans, after a bolus dose of 34 mg aspartame/kg body weight, plasma phenylalanine levels rise to a peak of 110 µM (approximately 11 µmol/dL plasma) (Dews, 1987; Stegink, 1987). An aspartame dose of 100 mg/kg in normal subjects increased fasting levels of phenylalanine from 54 µM to 202 µM (Stegink *et al.,* 1980).

The aspartame plasma level of phenylalanine in infant monkeys 1 hour after an oral dose of 2 g aspartame/kg body weight was approximately 82 µmol/dL plasma (Reynolds *et al.,* 1980). Mean plasma levels of phenylalanine 1 hour after an oral dose of 0.5, 0.75, or 1 g/kg were 162, 241, or 442 nmol/mL plasma, respectively, in Sprague-Dawley rats and 192, 147, or 312 nmol/mL plasma in CD-1® mice (Diomede *et al.,* 1991).

TOXICITY

Experimental Animals

Aspartame is reported to have low toxicity in experimental model systems. The oral LD_{50} of aspartame in rats and mice is reported to be more than 10 g/kg per day (RTECS, 2002).

In a 90-day oral gavage study in F344/N rats, doses up to 1,000 mg aspartame/kg body weight were not proconvulsant (Tilson *et al.,* 1989). Aspartame at 1,000 mg/kg had no effect on learning and memory in F344/N rats (Tilson *et al.,* 1991). Aspartame at doses up to 1,000 mg/kg given orally to male Sprague-Dawley and F344/N rats also did not alter levels of norepinephrine, dopamine, or serotonin in the brain at intervals up to 240 minutes after administration (Freeman *et al.,* 1990). Other studies have also revealed that oral administration of aspartame at doses up to 704 mg/kg did not affect the behavior of Sprague-Dawley rats when evaluated by spontaneous activity or "wheel running" (Holder and Yirmiya, 1989).

The toxicity of aspartame was evaluated in male and female Wistar rats fed diets that delivered approximately 0, 1, 2, or 4 g aspartame/kg body weight daily from 6 weeks of age up to 104 weeks of age (Ishii *et al.,* 1981). Each group contained 60 animals, with additional animals added for interim sacrifice at 26 or 52 weeks. Dose-dependent decreases in body weight gain and dose-related increases in urinary calcium occurred in the 2 and 4 g/kg groups. Clinical chemistry and hematologic endpoints were measured at the interim and terminal sacrifices, and no treatment-related changes

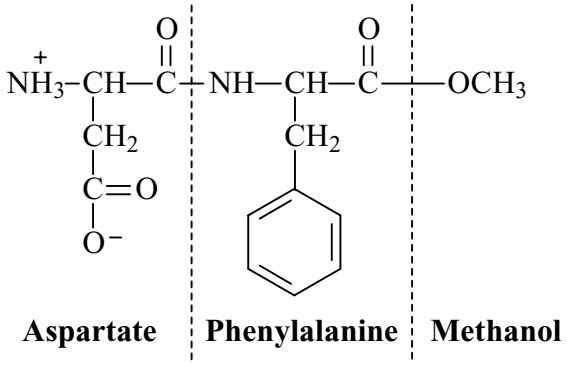


FIGURE 1
Chemical Structure of Aspartame with its Components Indicated by the Dotted Lines (Stegink, 1987)

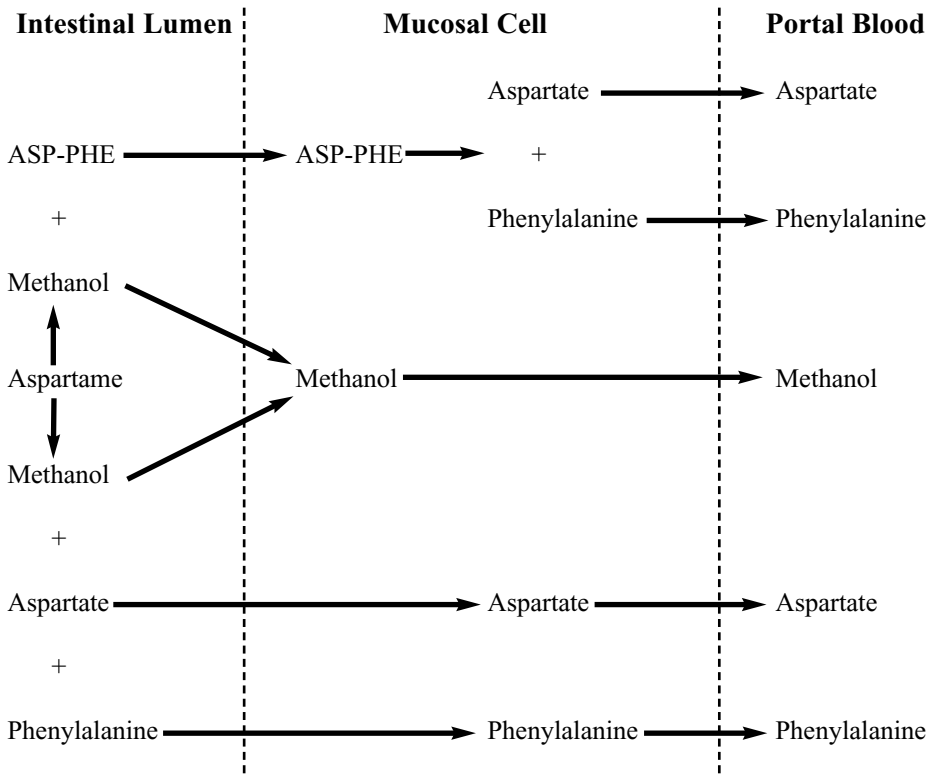


FIGURE 2
Hydrolysis of Aspartame (Stegink, 1987)

were noted except for a decrease in total cholesterol in the 4 g/kg group at terminal sacrifice. The study indicated that portions of major organs and gross lesions were examined microscopically, although complete details of this evaluation were not given.

Humans

There have been no large-scale, controlled studies to evaluate whether aspartame causes toxic effects in humans; however, the FDA has a passive surveillance system for reporting side effects from aspartame use (CDC, 1984; Bradstock *et al.,* 1986; Tollefson, 1988; Tollefson and Barnard, 1992). Generally, passive surveillance systems receive reports from consumers, and the types of side effects reported are anecdotal in nature and usually are conditions that occur immediately after consumption of a product (CDC, 1984).

The FDA has collected information on adverse health effects reported by individuals through its Adverse Reaction Monitoring system (ARMS). In 1988, the FDA presented an analyses of the aspartame adverse reports (Tollefson, 1988). As of 1988, the FDA received approximately 4,000 consumer complaints, and the most common adverse reactions were headaches (19%), dizziness/balance problems (8%), change in mood (7%), and vomiting and nausea (7%); a variety of other symptoms were also reported. Based on this information, it is not possible to determine the incidence or severity of the reported effect, and it is not possible to determine if the reaction is truly associated with the intake of aspartame (Tollefson, 1988). In 1992, the FDA reported that it received 251 reports of seizures after aspartame ingestion, but it concluded that the information did not support the claim that the seizures were related to aspartame consumption (Tollefson and Barnard, 1992).

The Centers for Disease Control (CDC) published an evaluation of reactions to aspartame and noted that neurological/behavioral symptoms were most frequently (67%) reported; however, the CDC concluded that it could not make a clear association between aspartame consumption and adverse effects (Bradstock *et al.,* 1986). The NutraSweet Company also collected postmarketing information on aspartame and reported that no evidence of a relationship between aspartame and adverse health effects was found (Butchko and Stargel, 2001).

Several studies in the literature have evaluated a small number of people in an attempt to gain some additional information on side effects from aspartame intake. Sixteen adults and two children, who reported to the FDA that they had seizures associated with aspartame consumption, were subsequently evaluated in a clinical study (Rowan *et al.,* 1995). In this evaluation, aspartame (50 mg/kg) or placebo was randomly administered to two groups in divided doses on days 2 and 4. No clinical seizures or other adverse effects were observed; however, plasma phenylalanine concentrations increased after aspartame ingestion (83.6 μM) compared to placebo (52.3 μM).

A double-blind controlled trial divided a group of 25 normal preschool children and a group of 23 children described as being sensitive to sugar into three groups that received one of three diets daily for 3 weeks: a diet that contained 5,600 mg sucrose/kg body weight and no artificial sweeteners; a diet low in sucrose and containing 38 mg aspartame/kg body weight; or a diet low in sucrose and containing 12 mg saccharin/kg body weight (Wolraich *et al.,* 1994). None of the diets affected the children's behavior or cognitive function.

Thirty-two subjects received approximately 30 mg aspartame/kg body weight per day or placebo for 7 days in a two-treatment, four period crossover design (Van en Eeden *et al.,* 1994). Thirty-three percent of the subjects that received aspartame reported headaches versus 24% in the placebo group. The authors concluded that some people were susceptible to headaches after aspartame consumption. Another study with 48 volunteers who received aspartame (up to 48 mg/kg per day for 20 days) or placebo found no neuropsychologic, neurophysiologic, or behavior effects in the aspartame group (Spiers *et al.,* 1998).

A variety of other studies have explored the effects of aspartame. One study reported that injection of 250 mg aspartame into healthy human subjects could lead to increased levels of urinary calcium but had no effect on plasma insulin or glucose levels (Nguyen *et al.,* 1998).

After a review of the data, the FDA concluded that there were no consistent patterns of symptoms attributed to the use of aspartame in humans. The FDA pointed out that people with phenylketonuria do not have the ability to metabolize the amino acid, phenylalanine, and should not use aspartame (FDA, 1994).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

A series of studies were conducted to evaluate the reproductive and developmental toxicity of aspartame in animals, and none found any evidence of these toxicities. Aspartame (500, 1,000, 2,000, or 4,000 mg/kg) was administered to CF-l mice on days 15 to 18 of gestation by oral gavage, and no adverse effects on maternal body weight, gestation length, reproductive indices, or litter size were noted (McAnulty *et al.,* 1989). In the pups, body weights, negative geotaxis, and righting reflexes were not altered, and there were no changes in time of eye opening or reflex pupil closure.

Searle & Company (Searle) conducted two-generation reproductive, perinatal, and postnatal toxicity studies in rats given up to 4,000 mg/kg aspartame, and there were no delays in the onset of developmental milestones in the dams (Molinary, 1984). However, no data from the study were presented in the review article, and there was no information on levels of aspartame metabolites in the dams or pups.

When adult female Sprague-Dawley rats were administered aspartame in the drinking water at concentrations up to 0.9% from 12 days prior to conception until pups were 38 days old, no effects on perinatal development were detected in the pups (Holder, 1989). This included two measures of morphologic development (latencies to pinnae detachment and eye opening) and two tests of development (latencies for surface righting at 7 days of age and negative geotaxis at 8 days of age). The estimated dose was up to 1,614 mg/kg per day in the dams and up to 3,566 mg/kg per day in the pups.

A 6% aspartame concentration given to pregnant rabbits in the diet from day 6 to day 16 or 20 had no effect on fetal weight or litter size (Ranney *et al.,* 1975). When pregnant guinea pigs were administered 500 mg/kg aspartame daily by oral gavage from day of conception to parturition, there were no effects on maternal weight gain, litter size, or pup birth weight (Dow-Edwards *et al.,* 1989); however, there was some effect on odor-associative learning in 15-day-old pups.

Up to 3,000 mg/kg aspartame given to infant macaque monkeys in the diet had no effect on growth rate, development milestones, or learning or sensory discrimination (Reynolds *et al.,* 1984). There were also no effects on long-term behavior in these primates (Suomi, 1984).

Humans

No reproductive or developmental toxicity studies of aspartame in humans were found in the literature.

CARCINOGENICITY

Experimental Animals

The FDA approved the use of aspartame in 1981 after reviewing studies submitted by Searle (*Fed. Regist.,* 1981a,b); the FDA commissioner's decision on the use of aspartame contains information on Searle's studies to determine any carcinogenic potential of aspartame, however, the experimental details of the studies were not published.

The FDA summary notes that prior to the approval, it established a Public Board of Inquiry to answer questions on aspartame including whether the ingestion of aspartame contributed to mental retardation, brain damage, or undesirable effects on neuroendocrine regulatory systems and whether the ingestion of aspartame might induce brain neoplasms in rats. The Board of Inquiry found that aspartame consumption would not pose an increased risk of adverse neurological effects. However, the Board did find that the available data were not conclusive to rule out an effect on the incidence of brain tumors in laboratory rats (*Fed. Regist.,* 1981a,b). In its final ruling, the FDA concluded that aspartame was safe for its proposed uses.

The FDA summarized a Searle 104-week study in Sprague-Dawley rats (*Fed. Regist.,* 1981a,b). In this study, 1, 2, 4, or 6 to 8 g/kg aspartame were given to groups of 40 male and 40 female rats in the diet; dosing began after weaning. Groups of 60 male and 60 female rats served as controls. Further details on this study were also reported in an aspartame monograph (Molinary, 1984); there were reductions in mean body weight and mean feed consumption in the 6 to 8 mg/kg groups, 2-year survival was poor (attributed to

spontaneous disease), and no treatment-related hematologic or clinical chemistry findings were reported. The Board of Inquiry was concerned that there was an occurrence of gliomas at a relatively early age in some of the exposed groups. A total of seven brain tumors occurred in exposed males (1 g/kg, 2/40; 2 g/kg, 1/40; 4 g/kg, 4/40; 6 to 8 g/kg, 0/40), and a total of five occurred in exposed females (2/40, 0/40, 1/40, 2/40; Cornell *et al.,* 1984). In an initial review, no brain tumors were observed in controls, but in subsequent review one occurred in a control male. There were brain tumor diagnosis inconsistencies among the various groups that reviewed these lesions (Koestner, 1984).

The FDA also reported on a two-generation study in Sprague-Dawley rats given 0, 2, or 4 g aspartame/kg body weight in the diet *in utero,* during lactation, and then for 104 weeks. Brain tumors occurred in three control males and one control female, in two males and one female in the 2 g/kg groups, and in one male and one female in the 4 g/kg groups. There were increased incidences of hyperplastic nodules of the liver in treated females; however, there were no treatment-related differences in neoplasm rates between control and treated groups (*Fed. Regist.,* 1981a,b; Molinary, 1984).

A principal product of aspartame decomposition is diketopiperizine. The FDA summarized a 115-week study of diketopiperizine in Sprague-Dawley rats exposed to 0, 0.75, 1.5, or 3.0 g aspartame/kg body weight; exposure began after weaning. There was no treatment-related carcinogenicity in that study, and there was none in a similar study in mice (*Fed. Regist.,* 1981a,b; Molinary, 1984).

The FDA also reported that a chronic aspartame mouse study did not show any treatment-related carcinogenic effects (*Fed. Regist.,* 1981a,b). Further details on this study were reported by Molinary (1984). Groups of 36 male and 36 female mice were fed 1, 2, or 4 g/kg aspartame for up to 110 weeks. Mean body weights of treated animals were similar to those of the controls, although feed consumption decreased with increasing aspartame concentration. There were no treatment-related effects on survival or behavior, there were no statistically significant differences in hematologic or clinical chemistry endpoints, and there were no treatment-related gross or microscopic findings.

An aspartame carcinogenesis study was also conducted in Japan (Ishii, 1981; Ishii *et al.,* 1981). In this study, 1, 2, or 4 g/kg aspartame or a 3:1 ratio of 4 g/kg aspartame:diketopiperazine metabolite (5-benzyl-3,6-dioxo-2-piperazine acetic acid) in the diet were administered to male and female Wistar rats. One atypical astrocytoma was found in a control rat, and two astrocytomas, two oligodendrogliomas and one ependymoma were found in the exposed groups. The authors concluded that there were no significant differences in the incidences of brain tumors between the control and treated groups. Complete experimental details of this study were not published.

In a 106-week study, dogs were fed 0, 1, 2, or 4 g/kg aspartame daily in the diet. Aspartame caused no treatment-related carcinogenic effects, although no experimental data were presented in the review article (Molinary, 1984).

Humans

There have been no large-scale epidemiology studies to evaluate the relationship between aspartame intake and cancer development in humans.

One study reported that the incidence of brain tumors in the United States increased between 1970 and 1980, and suggested that while the cause of the increase remains unknown, environmental factors, including aspartame use might have contributed to this increase (Olney *et al.,* 1996). Others have concluded that diagnostic advances or other factors could better account for these findings (Levy and Hedeker, 1996; Gurney *et al*., 1997; Smith *et al*., 1998).

GENETIC TOXICITY

There are few published mutagenicity data for aspartame. Aspartame was reported to be negative in a *Salmonella* mutagenicity test using strains TA100 and TA98, but following nitrosation (40 mM nitrite under acidic pH at 37° C), mutagenic activity was demonstrated by the resulting product (aspartame concentration of 8 mM in the reaction product) in TA100, TA104, and TA98 in the absence of S9 metabolic activation enzymes (Shephard *et al.,* 1993); addition of 10% S9 reduced the magnitude of the mutagenic response in TA100, but did not eliminate it. No mutagenicity was detected in TA98

with aspartame in the presence of S9. The nitrosated product was also tested in TA102 without S9, but no mutagenic activity was detected. The authors concluded from kinetic studies that nitrosation of the primary amine produced the mutagenic product, and they suggested that under naturally occurring conditions in the stomach, side chain nitrosation would be the primary pathway of activity. Thus, the mutagenic products produced through this experimental nitrosation procedure would not likely be produced through normal digestive reactions *in vivo*. Results of an assay to measure induction of unscheduled DNA synthesis in rat hepatocytes treated with aspartame *in vitro* were negative, indicating the absence of induced DNA damage by aspartame (Jeffrey and Williams, 2000). *In vivo,* a mixture of aspartame (up to 350 mg/kg) and a second sweetener, acesulfame potassium (up to 150 mg/kg), was reported to be negative in a test for induction of chromosomal aberrations in bone marrow cells of male Swiss mice when administered by gavage (Mukhopadhyay *et al.,* 2000). A dose-related increase in the percentage of cells with chromosomal aberrations was noted with increasing doses of the two sweeteners, but the increase was not statistically significant.

Background on Genetically Altered Mice

Mutation and/or deletions of tumor suppressor genes or activation of protooncogenes can disrupt cell function and predispose an animal to cancer. In the current studies, three genetically altered mouse models with either a loss of heterozygosity in a critical cancer gene (Trp53 or Cdkn2a) or a gain of oncogene function (Ha-*ras*) were used to determine how these animals would response to aspartame exposure. Two of these mouse models have been shown to be susceptible to the rapid development of cancer. The Tg.AC hemizygous and p53 haploinsufficient mice are being evaluated by the National Institute of Environmental Health Sciences (NIEHS) as models for identifying chemical toxicity and/or chemical carcinogenic processes (Tennant *et al.,* 1996).

FVB/N-TgN(v-Ha-ras)Led (Tg.AC) Hemizygous Mouse Model

The Tg.AC mouse (on an FVB/N background) was developed by Leder *et al.* (1990) by introduction via pronuclear injection of a tripartite transgene composed of the promotor of the mouse embryonic *zeta*-globin gene, through the v-Ha-*ras* coding sequence, with point mutation in codons 12 and 59, and an SV40 polyadenylation sequence.

The Tg.AC transgenic mouse model has been evaluated as a reporter phenotype (skin papillomas) in response to either genotoxic or nongenotoxic carcinogens, including tumor promoters (Spalding *et al.,* 1993, 1999; Tennant *et al.,* 1999). Tg.AC mice are hemizygous for a mutant v-Ha-*ras* transgene. The model was developed by Leder *et al.* (1990) with an inducible zeta-globin promoter driving the expression of a mutated v-Ha-*ras* oncogene and is regarded as a genetically initiated model. With the exception of bone marrow, constitutive expression of the transgene cannot be detected in adult tissues. The transgene is usually transcriptionally silent until activated by certain treatments including full-thickness wounding, ultraviolet irradiation, or exposure to some chemicals (Cannon *et al.,* 1997; Trempus *et al.,* 1998). The Tg.AC hemizygous mouse develops a high incidence of skin papillomas in response to topical application of 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA), and TPA has been used as a positive control in NIEHS Tg.AC mouse studies (Spalding *et al.,* 1993). Point mutations in the Ha-*ras* gene are believed to be early events in the induction of skin papillomas and malignancies. Topical application of carcinogens to the shaved dorsal surface of Tg.AC mice induces epidermal squamous cell papillomas or carcinomas, a reporter phenotype that defines the activity of the chemical. The oral route of administration can also generate tumor responses in the skin of Tg.AC mice and lead to squamous cell papillomas and/or carcinomas of the forestomach. To date, the appearance of either spontaneous or induced tumors has been shown to require activation of transgene expression. However, the mechanism of response by the Tg.AC model to chemical carcinogens is not yet understood.

In NIEHS studies, mice are exposed beginning at 2 months of age for a total of 6 to 9 months. Incidences of cutaneous papillomas at various sites have been reported at 3.7% and 3.8% in 26-week-old control male and female Tg.AC mice, respectively (Mahler *et al.,* 1998). Cutaneous papillomas occurring at sites such as the lip, pinnae, prepuce, and vulva suggest a possible relationship to grooming and chronic irritation. Up to 32% of Tg.AC homozygous and heterozygous male or female mice can develop odontogenic tumors as early as 26 weeks (Wright *et al.,* 1995; Mahler *et al.,* 1998). A

number of different tumor types occur in untreated Tg.AC hemizygous mice at incidences greater than 3% including odontogenic tumors, forestomach papillomas, cutaneous papillomas, alveolar/bronchiolar adenomas, salivary gland duct carcinomas, and erythroleukemia (Mahler *et al.,* 1998). In the FVB mouse (the background strain for the Tg.AC hemizygous mouse), alveolar/bronchiolar neoplasms occur at 14 months of age (Mahler *et al.,* 1996).

The Tg.AC hemizygous mouse model was used in the current Report for the studies of aspartame and in a companion Report for the studies of acesulfame potassium (NTP, 2005) because this model has been reported to detect both nongenotoxic and genotoxic carcinogens (Spalding *et al.,* 1993; Tennant *et al.,* 1995*,* 1996; Pritchard, 2003).

B6.129-Trp53^{tm1Brd} (N5) Haploinsufficient Mouse Model

The heterozygous B6.129-Trp53 (N12)$^{tmlBrd(+/–)}$ mouse (on a B6.129S7 background) was developed by Donehower *et al.* (1992). A null mutation was introduced into one p53 allele by homologous recombination in murine embryonic stem cells. Insertion of a neo cassette resulted in deletion of a 450-base pair gene fragment containing 106 nucleotides of exon 5 and approximately 350 nucleotides of intron 4.

Trp53, a nuclear protein, plays an essential role in the regulation of the cell cycle, specifically in the transition from G_0 to G_1, as well as G_2 to M, and the spindle apparatus. The p53 protein has a short half-life and exists at a very low concentration under normal cell physiological conditions. However, in DNA damaged cells that are able to replicate, p53 is expressed in high amounts with a significant increase in half-life due to post-translational modification (phosphorylation or acetylation). Mutations in p53 may also increase the protein half-life and alter functions that may contribute to transformation and development of the malignant phenotype. p53 is a DNA-binding protein containing DNA-binding, oligomerization, and transcription activation domains. Many amino acid residues in different p53 domains may be phosphorylated or acetylated, which may determine specific p53 functions. It is postulated to bind as a tetramer to a p53-binding site and activate expression of downstream genes that inhibit growth and/or invasion or promote apoptosis, functioning as a tumor suppressor. This protein is critical to tumor suppression in humans and rodents. Mutants of p53 that fail to bind the consensus DNA binding site, and hence are unable to function as tumor suppressors, frequently occur in human cancers. Alterations of the Tp53 gene occur not only as somatic mutations in human malignancies, but also as germline mutations in some cancer-prone families with Li-Fraumeni syndrome.

The mouse heterozygous for a p53 null allele (+/–) has only a single functional wild-type p53 allele which provides a target for mutagens. The p53 tumor suppressor gene is one of the most common sites for mutations and gene alterations in human cancer (Harris, 1996a,b,c).

Heterozygous p53$^{(+/–)}$ transgenic mice develop normally, and like humans and other mammals, develop cancer (primarily lymphomas or sarcomas) with age, but often with decreased latency and increased susceptibility.

B6.129-Cdkn2a^{tm1Rdp} (N2) Deficient Mouse Model

A transgenic model with a targeted deletion in the Cdkn2a locus (Cdkn2a deletion), and thus disruption in p16^{Ink4a} and p19^{Arf} function, was developed by Serrano *et al.* (1996). The genetic background of this mouse is derived from parental strains C57BL/6 and 129WW6 (75%), 129/Sv (20%), and 129SJL (5%).

The Cdkn2a gene generates several different transcript variants using different first exons and alternate polyadenylation sites. The p16^{Ink4a} variants encode structurally related protein isoforms that function to inhibit CDK4 kinase. The p19^{Arf} transcript includes an alternate first exon located upstream of the remainder of the gene. This alternate open reading frame (ARF) specifies a protein which is structurally unrelated to the other variants. The p19^{Arf} product functions to stabilize the tumor suppressor protein, p53. The p19^{Arf} binds Mdm2, a protein that targets degradation of p53 through ubiquination. Both the p16^{Ink4a} CDK4 inhibitors and the p19^{Arf} protein encoded by the Cdkn2a gene play a critical role in G_1/S progression of the cell cycle.

Transition from G_1 to S phase in the mammalian cell cycle is under complex regulatory control, and one G_1/S regulatory pathway involves p16 protein (encoded by the Cdkn2a gene). p16 inhibits the CDK4/cyclin D1 complex, preventing CDK4 from phosphorylating pRb, ensuring that pRb maintains G_1 arrest. Disruption of this

pathway by p16 gene mutations perturbs the cell cycle (Reed *et al.,* 1996), and in the case of the Cdkn2a knockout mice, allows more cell proliferation to occur.

This gene is frequently mutated or deleted in a wide variety of tumors and is known to be an important tumor suppressor gene. Genetic alterations in the Cdkn2a gene play an important role in human cancers, particularly with brain tumor carcinogens (Liggett and Sidransky, 1998). The Cdkn2a gene encodes two distinct proteins, the $p16^{Ink4a}$ protein, which is a component of the retinoblastoma (Rb) gene pathway, and the $p19^{Arf}$ protein (or in humans, the $p14^{Arf}$ protein), which interacts with Mdm2 and neutralizes Mdm2 inhibition of p53 (Pomerantz *et al.,* 1998).

Homozygous null $Cdkn2a^{(-/-)}$ (i.e., $p16^{Ink4a(-/-)}$ and $p19^{Arf(-/-)}$) are viable and fertile. On inspection, these mice appear normal until approximately 2 months of age, but histologic analysis of the spleen shows a mild proliferative expansion of the white pulp and the presence of numerous megakaryocytes and lymphoblasts in the red pulp. The mice homozygous for Cdkn2a null alleles (–/–) develop tumors at an average age of 29 weeks, and most mice die by 36 weeks. Lymphomas and fibrosarcomas are two common types of tumors seen in these mice. In contrast, the $Cdkn2a^{(+/-)}$ mouse does not usually develop obvious tumors or display compromised health until after 36 weeks of age (Serrano *et al.,* 1993)

At this time, the NIEHS and the NTP do not have enough information on the characteristics of Cdkn2a deficient mice to predict how this model might respond to different types of carcinogens.

Study Rationale

Aspartame has been evaluated in conventional rodent cancer studies (non-NTP studies) and is considered negative for carcinogenicity. It has been argued that those studies showed a slight increase in brain tumors. Because of these uncertainties, aspartame was selected by the NIEHS for evaluation of its toxicologic and carcinogenic potential.

The conventional rodent bioassay has been used for over three decades and is credible in identifying carcinogens thought to pose risks to humans (Tomatis *et al.,* 1997), although only two chemicals in rats and one in mice of over 500 chemicals studied by the NTP have shown unequivocal increases in brain tumors. An ongoing goal of the NIEHS and the NTP is to seek other model systems for toxicology and carcinogenesis studies, especially those that can provide mechanistic information that will assist in understanding an agent's mode of action. The use of genetically altered models holds promise for improving the accuracy and efficacy of experimental assessment of the carcinogenic potential of chemicals. Genetically altered mouse models carry activated oncogenes or inactivated tumor suppressor genes known to be involved in neoplastic processes in humans and rodents. This trait may allow them to respond to carcinogens more quickly than conventional rodent strains. In addition, the neoplastic effects of agents can be observed in genetically altered models within a time frame in which few, if any, spontaneous tumors would arise. The high incidences of spontaneous or background tumors, which occur most often late in the 2-year rodent studies, can hinder interpretation of the findings and their implications for human health. The use of target or reporter genes allows for direct molecular and cellular analysis of a chemical's effects in genetically modified mouse models and can provide additional mechanistic information about the mode of action.

For the past few years, the NIEHS and the NTP have been actively evaluating genetically altered strains in toxicologic testing strategies. Based on completed evaluations, three models, the Tg.AC hemizygous (v-Ha-*ras*) and p53-deficient (p53 haploinsufficient), and the *ras* H2 (cHa-*ras*-transgene) mice have shown potential usefulness in identifying carcinogens (Pritchard *et al.,* 2003). Aspartame was one of the test agents selected for the continued evaluation of the genetically modified Tg.AC hemizygous and p53 haploinsufficient strains and for initial evaluations of the Cdkn2a deficient mouse model. The vast majority of studies utilizing genetically modified mice for cancer hazard identification have employed a dosing duration of 6 months, after which mice are examined for tumor development. Although this may be adequate to identify relatively potent carcinogens, there is uncertainty over the adequacy of this dosing duration to produce a tumor response with weaker carcinogens. To partially address this question, the current studies were carried out for 9 months rather than 6 months.

MATERIALS AND METHODS

Procurement and Characterization of Aspartame

Aspartame was obtained from Spectrum Quality Products, Inc. (Gardena, CA) in two shipments of one lot (8415-14-02 RTI) used for the 9-month studies in Tg.AC hemizygous, p53 haploinsufficient, and Cdkn2a deficient mice. Identity and purity analyses were conducted by the analytical chemistry laboratory, Research Triangle Institute (Research Triangle Park, NC) and the study laboratory (BioReliance Corporation, Rockville, MD); the study laboratory also conducted stability analyses. Reports on analyses performed in support of the aspartame studies are on file at the National Institute of Environmental Health Sciences.

Lot 8415-14-02 RTI of the chemical, a white, odorless, crystalline powder, was identified as aspartame by the analytical chemistry laboratory using infrared and proton nuclear magnetic resonance spectroscopy and by the study laboratory using infrared spectroscopy. All spectra were consistent with the structure of aspartame, and the infrared spectra matched a reference spectrum (*Aldrich*, 1981) of aspartame.

The purity of lot 8415-14-02 RTI was determined by the analytical chemistry laboratory using high-performance liquid chromatography (HPLC). The study laboratory confirmed the purity using HPLC and comparison to a reference sample of the same lot.

For lot 8415-14-02 RTI, HPLC by the analytical chemistry laboratory indicated one major peak and three impurities with a combined area of 0.5% relative to the total peak area. HPLC by the study laboratory indicated a purity of 98.7% for lot 8415-14-02 RTI relative to the reference. The overall purity of lot 8415-14-02 RTI was determined to be greater than 98%.

To ensure stability, the bulk chemical was stored at room temperature, protected from light, in polyethylene bags inside metal drums. The stability of the bulk chemical was monitored during the studies by the study laboratory using HPLC. No degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared every 2 weeks by mixing aspartame with feed (Table I1). Homogeneity and stability studies of dose formulations were performed by the analytical chemistry laboratory and the study laboratory using HPLC. Homogeneity was confirmed for 1,000, 10,000, and 50,000 ppm formulations by the analytical chemistry laboratory, and for 3,125 and 50,000 ppm formulations by the study laboratory. Stability was confirmed for 1,000 and 10,000 ppm formulations by the analytical laboratory for up to 28 days at –20° C and 5° C for dose formulations stored in doubled polyethylene bags. There was no significant loss of aspartame from NTP-2000 feed formulations under simulated animal room conditions.

Periodic analyses of the dose formulations of aspartame were conducted by the study laboratory using HPLC. During the 9-month studies, the dose formulations were analyzed seven times; all of the dose formulations analyzed were within 10% of the target concentrations (Table I2). Animal room samples of these dose formulations were also analyzed, and 27 of 35 animal room samples were within 10% of the target concentrations (Table I2). Dose formulations were stored refrigerated, protected from light, in doubled polyethylene bags for up to 28 days.

9-Month Studies

Study Design

Groups of 15 male and 15 female mice were fed diets containing 0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm aspartame for 40 weeks.

Source and Specification of Animals

Male and female FVB/N-TgN(v-Ha-*ras*)Led (Tg.AC) hemizygous, B6.129-*Trp53*^{tm1Brd} (N5) haploinsufficient, and B6.129-Cdkn2a^{tm1Rdp} (N2) deficient mice were obtained from Taconic Farms (Germantown, NY) for use in the 9-month studies. Tg.AC hemizygous mice were quarantined for 11 days, p53 haploinsufficient mice were quarantined for 14 days, and Cdkn2a deficient mice were quarantined for 27 days before the beginning of the studies. Tg.AC hemizygous mice were approximately 6 weeks old, p53 haploinsufficient mice were approximately 7 weeks old, and Cdkn2a deficient mice were 7 to 9 weeks old at the beginning of the studies. Five male and five female mice were randomly selected for parasite evaluation and gross observation of disease. Blood was collected from five male and five female mice of each strain at the beginning of the studies. The sera were analyzed for antibody titers to rodent viruses (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b). All results were negative.

Animal Maintenance

Mice were housed individually. Feed and water were available *ad libitum*, and feed consumption was measured weekly. The feed was irradiated to reduce potential microbial contamination. Cages were changed weekly, and racks were changed and rotated every 2 weeks. Further details of animal maintenance are given in Table 1.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings and body weights were recorded initially, weekly, and at the end of the studies.

Complete necropsies and microscopic examinations were performed on control and 50,000 ppm mice. At necropsy, the brain, heart, right kidney, liver, lungs, right testis, and thymus were weighed. All organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. Upon completion of the laboratory pathologist's histologic evaluation, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment (QA) was performed. The QA pathologist reviewed all neoplasms from all groups and all slides from all animals in the control and high dose groups in Tg.AC hemizygous, p53 haploinsufficient, and Cdkn2a deficient mice. Results were reviewed by the NTP Pathology Working Group (PWG) Chairperson to address any inconsistencies in the diagnoses made by the laboratory and QA pathologists. The PWG chairperson presented representative histopathology slides containing examples of lesions potentially related to chemical administration, examples of disagreements in diagnoses between the laboratory and QA pathologists, and lesions of general interest to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. The testis and epididymis in Tg.AC hemizygous males were further reviewed in a supplemental quality assessment by the QA pathologist. These findings were in general agreement with those of the laboratory pathologist and were reviewed by an NTP pathologist. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, the reviewing pathologist(s), and the PWG. Details of the NTP review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.,* (1985).

TABLE 1
Experimental Design and Materials and Methods in the 9-Month Feed Studies of Aspartame

Tg.AC Hemizygous Mice	p53 Haploinsufficient Mice	Cdkn2a Deficient Mice
Study Laboratory BioReliance Corporation (Rockville, MD)	BioReliance Corporation (Rockville, MD)	BioReliance Corporation (Rockville, MD)
Strain FVB/N-TgN(v-Ha-*ras*)Led (Tg.Ac) hemizygous	B6.129-*Trp53*^{tm1Brd} (N5) haploinsufficient	B6.129-Cdkn2a^{tm1Rdp} (N2) deficient
Animal Source Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies 11 days	14 days	27 days
Average Age When Studies Began 6 weeks	7 weeks	7-9 weeks
Date of First Exposure October 11, 1999	October 14, 1999	October 27, 1999
Duration of Exposure 40 weeks	40 weeks	40 weeks
Date of Last Exposure July 11, 2000	July 17, 2000	July 28, 2000
Necropsy Dates July 10-11, 2000	July 13-17, 2000	July 26-28, 2000
Average Age at Necropsy 45 weeks	46 weeks	46-48 weeks
Size of Study Groups 15 males and 15 females	15 males and 15 females	15 males and 15 females
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as Tg.AC hemizygous mice	Same as Tg.AC hemizygous mice
Animals per Cage 1	1	1
Method of Animal Identification Tail tattoo	Tail tattoo	Tail tattoo
Diet Irradiated NTP-2000 open formula meal (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*	Same as Tg.AC hemizygous mice	Same as Tg.AC hemizygous mice

TABLE 1
Experimental Design and Materials and Methods in the 9-Month Feed Studies of Aspartame

Tg.AC Hemizygous Mice	p53 Haploinsufficient Mice	Cdkn2a Deficient Mice
Water Tap water (Washington Suburban Sanitary Commission Potomac Plant) via automatic watering system (Edstrom Industries, Inc. Waterford, WI), available *ad libitum*	Same as Tg.AC hemizygous mice	Same as Tg.AC hemizygous mice
Cages Polycarbonate cages (Lab Products, Inc., Seaford, DE), changed weekly	Same as Tg.AC hemizygous mice	Same as Tg.AC hemizygous mice
Bedding Irradiated Sani-Chips® (P.J. Murphy Forest Produces, Montville, NJ), changed weekly	Same as Tg.AC hemizygous mice	Same as Tg.AC hemizygous mice
Cage Filters Remay 2016 (Snow Filtration, West Chester, OH), changed every 2 weeks	Same as Tg.AC hemizygous mice	Same as Tg.AC hemizygous mice
Racks Stainless steel (Lab Products, Inc., Seaford, DE), changed and rotated every 2 weeks	Same as Tg.AC hemizygous mice	Same as Tg.AC hemizygous mice
Animal Room Environment Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Exposure Concentrations 0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm in feed, available *ad libitum*	0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm in feed, available *ad libitum*	0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm in feed, available *ad libitum*
Type and Frequency of Observation Observed twice daily; animals were weighed and clinical observations were recorded initially, weekly, and at the end of the studies. Feed consumption was recorded weekly.	Observed twice daily; animals were weighed and clinical observations were recorded initially, weekly, and at the end of the studies. Feed consumption was recorded weekly.	Observed twice daily; animals were weighed and clinical observations were recorded initially, weekly, and at the end of the studies. Feed consumption was recorded weekly.
Method of Sacrifice Carbon dioxide asphyxiation	Carbon dioxide asphyxiation	Carbon dioxide asphyxiation
Necropsy Necropsies were performed on all mice. Organs weighed were the brain, heart, right kidney, liver, lung, right testis, and thymus	Necropsies were performed on all mice. Organs weighed were the brain, heart, right kidney, liver, lung, right testis, and thymus	Necropsies were performed on all mice. Organs weighed were the brain, heart, right kidney, liver, lung, right testis, and thymus

TABLE 1
Experimental Design and Materials and Methods in the 9-Month Feed Studies of Aspartame

Tg.AC Hemizygous Mice	p53 Haploinsufficient Mice	Cdkn2a Deficient Mice
Histopathology		
Complete histopathology was performed on all control and 50,000 ppm mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eye, gallbladder, harderian gland, heart with aorta, kidney, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), larynx, liver, lung and mainstem bronchi, mammary gland (with mesenteric lymph nodes), nose, oral cavity, ovary, pancreas, parathyroid gland, preputial gland, prostate gland, salivary gland, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, tongue, trachea, uterus, vagina, and Zymbal's gland.	Complete histopathology was performed on all control and 50,000 ppm mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland,bone with marrow, brain, clitoral gland, esophagus, eye, gallbladder, harderian gland, heart with aorta, kidney, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), larynx, liver, lung and mainstem bronchi, mammary gland (with mesenteric lymph nodes), nose, oral cavity, ovary, pancreas, parathyroid gland, preputial gland, prostate gland, salivary gland, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, tongue, trachea, uterus, vagina, and Zymbal's gland.	Complete histopathology was performed on control and 50,000 ppm mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eye, gallbladder, harderian gland, heart with aorta, kidney, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), larynx, liver, lung and mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland, nose, oral cavity, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, tongue, trachea, urinary bladder, uterus, vagina, and Zymbal's gland.
Limited histopathology was performed on the 3,125, 6,250, 12,500, and 25,000 ppm mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, brain, heart with aorta, kidney, liver, lung and mainstem bronchi, mammary gland (with mesenteric lymph nodes), ovary, preputial gland, prostate gland, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, and uterus.	Limited histopathology was performed on the 3,125, 6,250, 12,500, and 25,000 ppm mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, brain, heart with aorta, kidney, liver, lung and mainstem bronchi, mammary gland (with mesenteric lymph nodes), ovary, prostate gland, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, and uterus.	Limited histopathology was performed on the 3,125, 6,250, 12,500, and 25,000 ppm mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, brain, heart, kidney, liver, lung,, lymph nodes (mandibular and mesenteric), mammary gland, ovary, pituitary gland, prostate gland, spleen, stomach (forestomach and glandular), testis, thymus, thyroid gland, and uterus.

STATISTICAL METHODS

Survival Analyses

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

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Appendixes A through F as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A3, B3, C3, D3, E3, and F3) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A3, B3, C3, D3, E3, and F3 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, to animals that do not reach terminal sacrifice.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal sacrifice; if the animal died prior to terminal sacrifice and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and $B6C3F_1$ mice (Portier *et al.,* 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

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

Analysis of Continuous Variables

Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973).

QUALITY ASSURANCE METHODS

The 9-month studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 9-month studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covered completeness and accuracy of

the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Report.

GENETIC TOXICOLOGY

The genetic toxicity of aspartame was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium,* micronucleated erythrocytes in mouse bone marrow, and increases in the frequency of micronucleated erythrocytes in mouse peripheral blood. The protocols for these studies and the results are given in Appendix G.

The genetic toxicity studies have evolved from an earlier effort by the NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term *in vitro* and *in vivo* genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity (Miller and Miller, 1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). However, it should be noted that not all cancers arise through genotoxic mechanisms. DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites (Ashby and Tennant, 1991). A positive response in the *Salmonella* test was shown to be the most predictive *in vitro* indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) (Tennant *et al.,* 1987; Zeiger *et al.,* 1990). Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

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

RESULTS

9-MONTH STUDY IN TG.AC HEMIZYGOUS MICE

Positive Control Tg.AC Hemizygous Mice

12-*O*-Tetradecanoylphorbol-13-acetate (TPA) (1.25 µg) was dermally administered to groups of 15 males and 15 females three times weekly for up to 29 weeks. Ninety-three percent of males and females developed more than 20 skin papillomas each by week 16 (data not shown). This is consistent with historical rates found in other studies (Tennant *et al.*, 2001).

Survival

Estimates of 9-month survival probabilities for male and female mice are shown in Table 2 and in the Kaplan-Meier survival curves (Figure 3). Survival of all exposed groups was similar to that of the control groups.

TABLE 2
Survival of Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Male						
Animals initially in study	15	15	15	15	15	15
Moribund	3	3	4	2	3	4
Natural deaths	3	0	3	1	1	1
Animals surviving to study termination	9	12	8	12	11	10
Percent probability of survival at end of study[a]	60	80	53	80	73	67
Mean survival (days)[b]	249	269	224	264	250	248
Survival analysis[c]	P=1.000N	P=0.362N	P=0.768	P=0.399N	P=0.771N	P=1.000N
Female						
Animals initially in study	15	15	15	15	15	15
Moribund	3	2	3	4	3	2
Natural deaths	1	3	3	2	1	5
Animals surviving to study termination	11	10	9	9	11	8
Percent probability of survival at end of study	73	67	60	60	73	53
Mean survival (days)	256	247	218	233	257	219
Survival analysis	P=0.454	P=0.916	P=0.541	P=0.596	P=1.000N	P=0.329

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice).
[c] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed group columns. A negative trend or lower mortality in an exposed group is indicated by **N**.

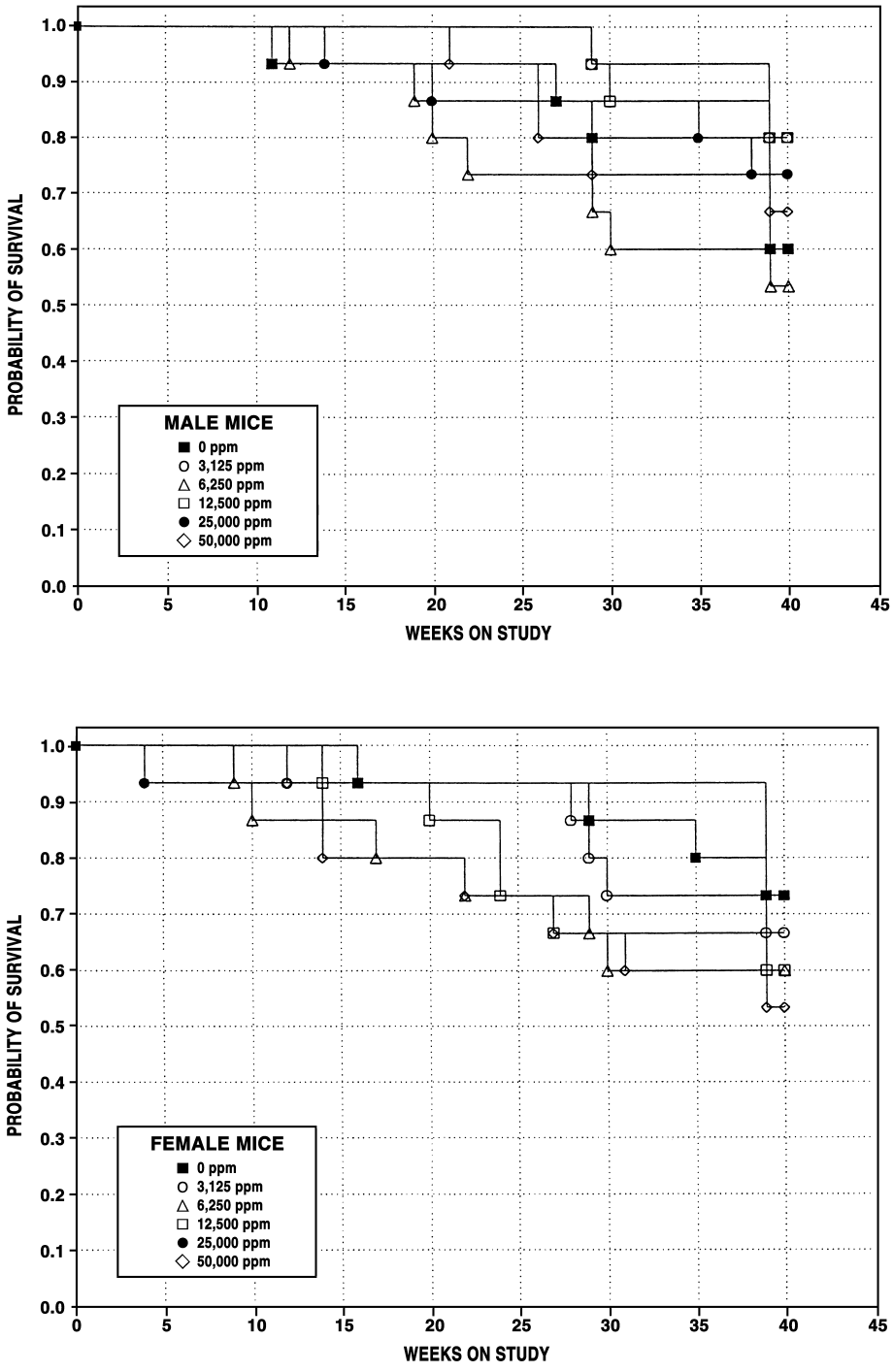


FIGURE 3
Kaplan-Meier Survival Curves for Male and Female Tg.AC Hemizygous Mice Exposed to Aspartame in Feed for 9 Months

Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of all exposed groups of males and of 3,125, 6,250, 12,500, and 25,000 ppm females were generally similar to those of the control groups throughout the study; mean body weights of 50,000 ppm females were greater than those of the controls after week 15 (Tables 3 and 4, and Figure 4). Feed consumption by the exposed groups was similar to that by the control groups (Tables J1 and J2). Dietary concentrations of 3,125, 6,250, 12,500, 25,000 and 50,000 ppm delivered average daily doses of approximately 490, 980, 1,960, 3,960 and 7,660 mg aspartame/kg body weight to males and 550, 1,100, 2,260, 4,420, and 8,180 mg/kg to females. Clinical findings included jaw masses, malocclusions, and cutaneous papillomas, but these findings were not considered related to aspartame exposure.

Organ Weights and Organ-Weight-to-Body-Weight Ratios

There was a significant increase in the absolute brain weight of 50,000 ppm males, and a significant increase in the relative brain weight of 25,000 ppm males (Table H1). Relative kidney weights were increased in 25,000 and 50,000 ppm males. Relative heart weights were also increased in some exposure groups but no exposure concentration-related response was apparent.

TABLE 3
Mean Body Weights and Survival of Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame

Weeks on Study	0 ppm		3,125 ppm			6,250 ppm			12,500 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	19.1	15	19.2	101	15	19.1	100	15	17.8	93	15
2	21.5	15	21.3	99	15	20.1	94	15	20.3	94	15
3	21.9	15	22.0	101	15	21.8	100	15	21.2	97	15
4	22.6	15	23.2	103	15	22.7	100	15	22.3	99	15
5	23.8	15	23.7	100	15	23.6	99	15	23.1	97	15
6	24.6	15	23.2	94	15	24.6	100	15	23.5	96	15
7	25.2	15	24.8	98	15	25.7	102	15	23.9	95	15
8	26.0	15	25.0	96	15	25.8	99	15	24.8	95	15
9	26.3	15	25.9	99	15	26.8	102	15	25.6	97	15
10	27.4	15	26.4	96	15	26.7	97	15	26.1	95	15
11	28.2	15	26.7	95	15	27.5	98	15	26.8	95	15
12	28.5	14	26.2	92	15	28.0	98	14	26.6	93	15
13	28.8	14	27.4	95	15	28.3	98	14	27.0	94	15
14	29.0	14	27.1	93	15	27.8	96	14	27.2	94	15
15	29.5	14	28.5	97	15	29.2	99	14	28.0	95	15
16	29.5	14	27.8	94	15	28.8	98	14	27.9	95	15
17	29.2	14	27.9	96	15	29.1	100	14	28.3	97	15
18	29.5	14	28.6	97	15	29.2	99	14	28.3	96	15
19	29.1	14	29.4	101	15	29.7	102	14	28.2	97	15
20	29.7	14	29.0	98	15	29.9	101	13	28.6	96	15
21	30.2	14	29.6	98	15	30.3	100	12	29.0	96	15
22	30.4	14	29.1	96	15	29.7	98	12	29.0	95	15
23	30.8	14	29.1	95	15	29.9	97	11	29.2	95	15
24	30.7	14	28.8	94	15	30.1	98	11	29.9	97	15
25	31.1	14	29.1	94	15	30.5	98	11	30.0	97	15
26	30.8	14	29.4	96	15	30.7	100	11	30.0	97	15
27	31.3	13	29.4	94	15	30.7	98	11	29.7	95	15
28	31.5	13	29.3	93	15	30.5	97	11	29.7	94	15
29	31.4	12	28.8	92	15	30.0	96	10	30.1	96	15
30	31.8	12	30.2	95	14	30.0	94	10	30.7	97	14
31	31.5	12	30.4	97	14	30.6	97	9	30.5	97	13
32	31.1	12	30.6	98	14	30.9	99	9	30.1	97	13
33	31.1	12	30.6	98	14	30.8	99	9	30.0	97	13
34	31.3	12	30.5	97	14	31.2	100	9	30.3	97	13
35	31.6	12	30.6	97	14	30.7	97	9	30.8	98	13
36	32.0	12	30.6	96	14	31.7	99	9	31.3	98	13
37	32.0	12	30.1	94	14	31.9	100	9	30.9	97	13
38	31.7	12	30.9	98	14	31.6	100	9	31.2	98	13
39	31.8	12	31.1	98	14	31.8	100	9	31.3	98	13
40	32.8	9	31.3	95	12	32.1	98	8	31.2	95	12
Mean for weeks											
1-13	24.9		24.2	97		24.7	99		23.8	96	
14-40	30.8		29.5	96		30.3	98		29.7	96	

TABLE 3
Mean Body Weights and Survival of Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame

Weeks on Study	25,000 ppm			50,000 ppm		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	19.1	100	15	18.6	97	15
2	21.7	101	15	20.8	97	15
3	22.1	101	15	22.0	101	15
4	23.3	103	15	22.7	100	15
5	23.9	100	15	23.6	99	15
6	25.0	102	15	23.8	97	15
7	25.4	101	15	24.9	99	15
8	25.9	100	15	24.6	95	15
9	26.1	99	15	25.5	97	15
10	26.4	96	15	26.2	96	15
11	27.5	98	15	26.8	95	15
12	26.7	94	15	27.0	95	15
13	27.9	97	15	27.4	95	15
14	28.1	97	15	27.6	95	15
15	28.5	97	14	28.1	95	15
16	28.4	96	14	27.8	94	15
17	29.0	99	14	28.6	98	15
18	28.8	98	14	28.6	97	15
19	29.3	101	14	28.4	98	15
20	29.9	101	14	28.9	97	15
21	29.6	98	13	29.4	97	14
22	29.4	97	13	28.5	94	14
23	29.7	96	13	28.4	92	14
24	30.0	98	13	27.9	91	14
25	29.6	95	13	28.0	90	14
26	30.3	98	13	27.2	88	14
27	30.5	97	13	29.0	93	12
28	30.7	98	13	29.7	94	12
29	30.9	98	13	30.0	96	12
30	31.1	98	13	30.9	97	11
31	31.0	98	13	30.9	98	11
32	31.3	101	13	31.0	100	11
33	31.1	100	13	31.2	100	11
34	30.8	98	13	30.5	97	11
35	30.3	96	13	31.1	98	11
36	29.8	93	12	31.6	99	11
37	29.7	93	12	31.2	98	11
38	30.3	96	12	31.3	99	11
39	31.7	100	11	31.2	98	11
40	31.2	95	11	31.7	97	10
Mean for weeks						
1-13	24.7	99		24.1	97	
14-40	30.0	97		29.6	96	

TABLE 4
Mean Body Weights and Survival of Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame

Weeks on Study	0 ppm		3,125 ppm			6,250 ppm			12,500 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	16.6	15	16.6	100	15	15.8	95	15	15.5	93	15
2	18.1	15	18.0	99	15	17.7	98	15	17.4	96	15
3	18.5	15	18.9	102	15	18.3	99	15	17.8	96	15
4	19.5	15	19.7	101	15	19.5	100	15	18.8	96	15
5	19.5	15	20.1	103	15	19.7	101	15	19.7	101	15
6	19.7	15	20.3	103	15	20.4	104	15	19.3	98	15
7	20.1	15	21.4	107	15	21.3	106	15	20.7	103	15
8	20.5	15	20.9	102	15	20.6	101	15	20.6	101	15
9	21.1	15	21.9	104	15	21.5	102	15	21.3	101	15
10	21.6	15	22.2	103	15	21.5	100	14	21.9	101	15
11	21.7	15	22.4	103	15	22.5	104	13	21.9	101	15
12	22.4	15	22.6	101	15	21.9	98	13	21.9	98	15
13	22.5	15	22.6	100	14	22.0	98	13	22.3	99	15
14	22.4	15	22.9	102	14	23.0	103	13	22.5	100	15
15	22.3	15	22.5	101	14	23.3	105	13	22.4	100	14
16	22.6	15	23.3	103	14	23.4	104	13	22.5	100	14
17	22.6	14	23.1	102	14	23.2	103	12	22.5	100	14
18	22.6	14	23.3	103	14	23.4	104	12	22.9	101	14
19	23.0	14	23.3	101	14	23.8	104	12	23.4	102	14
20	23.1	14	23.1	100	14	23.7	103	12	23.0	100	13
21	23.9	14	24.2	101	14	24.7	103	12	23.3	98	13
22	23.7	14	24.3	103	14	24.6	104	11	22.9	97	13
23	24.4	14	24.6	101	14	24.6	101	11	23.1	95	13
24	24.4	14	24.1	99	14	24.8	102	11	23.1	95	13
25	24.4	14	25.2	103	14	25.0	103	11	24.0	98	11
26	24.9	14	25.6	103	14	25.0	100	11	23.9	96	11
27	25.1	14	25.6	102	14	24.9	99	11	23.5	94	11
28	25.3	14	25.2	100	14	25.1	99	11	25.0	99	10
29	25.3	14	25.4	100	13	24.9	98	11	24.9	98	10
30	26.0	13	25.0	96	12	25.6	99	10	25.4	98	10
31	25.8	13	25.1	97	11	26.3	102	9	25.1	97	10
32	25.1	13	25.1	100	11	26.1	104	9	25.3	101	10
33	25.6	13	25.1	98	11	26.4	103	9	25.0	98	10
34	25.6	13	25.1	98	11	26.6	104	9	25.4	99	10
35	25.4	13	26.0	102	11	26.9	106	9	26.1	103	10
36	26.1	12	25.7	99	11	27.8	107	9	26.3	101	10
37	25.9	12	25.4	98	11	27.6	107	9	25.8	100	10
38	26.2	12	26.2	100	11	27.0	103	9	26.0	99	10
39	26.5	12	26.4	100	11	27.6	104	9	26.4	100	10
40	26.7	11	25.4	95	10	26.9	101	9	26.5	99	9
Mean for weeks											
1-13	20.1		20.6	102		20.2	100		19.9	99	
14-40	24.6		24.7	100		25.3	103		24.3	99	

TABLE 4
Mean Body Weights and Survival of Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame

Weeks on Study	25,000 ppm			50,000 ppm		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	16.4	99	15	16.6	100	15
2	18.1	100	15	17.8	98	15
3	17.6	95	15	17.9	97	15
4	19.6	101	15	19.6	101	15
5	20.1	103	14	20.3	104	15
6	20.2	103	14	20.2	103	15
7	20.8	104	14	21.3	106	15
8	21.1	103	14	20.7	101	15
9	21.6	102	14	21.8	103	15
10	21.9	101	14	22.4	104	15
11	22.5	104	14	22.7	105	15
12	21.7	97	14	23.2	104	15
13	23.2	103	14	23.1	103	14
14	22.9	102	14	23.1	103	12
15	23.6	106	14	23.5	105	12
16	23.4	104	14	24.5	108	12
17	23.8	105	14	24.4	108	12
18	23.7	105	14	24.5	108	12
19	23.6	103	14	24.9	108	12
20	23.7	103	14	25.9	112	12
21	24.1	101	14	26.0	109	12
22	24.2	102	14	25.7	108	12
23	23.9	98	14	25.7	105	11
24	24.3	100	14	26.1	107	11
25	24.4	100	14	26.2	107	11
26	24.7	99	14	26.1	105	11
27	24.9	99	14	26.3	105	11
28	25.1	99	14	27.3	108	10
29	25.0	99	14	27.5	109	10
30	24.8	95	14	27.2	105	10
31	24.4	95	14	27.9	108	10
32	24.3	97	14	28.0	112	9
33	24.6	96	14	27.8	109	9
34	24.7	97	14	27.9	109	9
35	25.3	100	14	27.7	109	9
36	25.0	96	14	27.8	107	9
37	24.7	95	14	27.2	105	9
38	24.6	94	14	28.1	107	9
39	24.8	94	14	28.0	106	9
40	26.5	99	11	28.3	106	8
Mean for weeks						
1-13	20.4	101		20.6	102	
14-40	24.4	98		26.4	107	

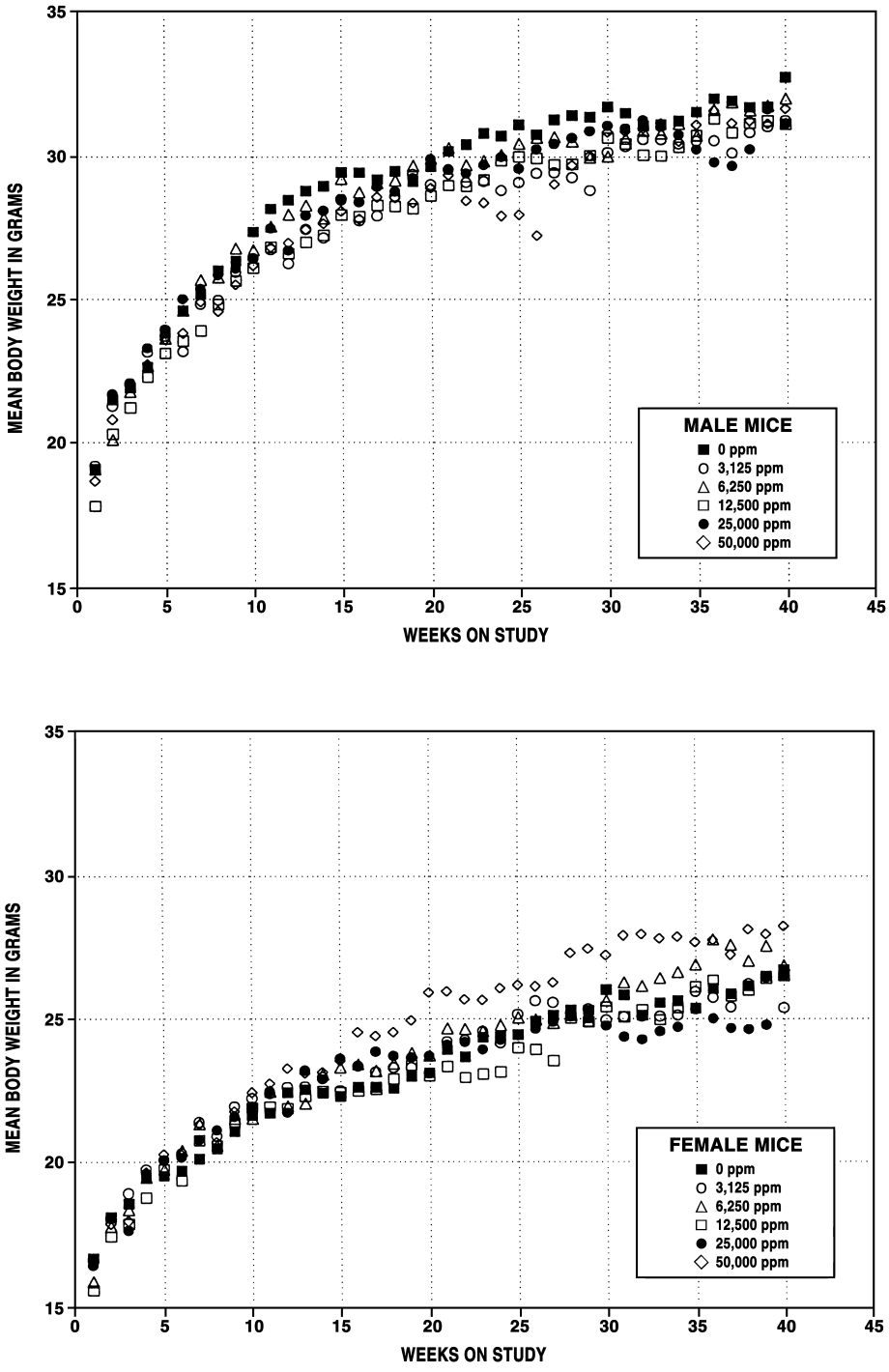


FIGURE 4
Growth Curves for Male and Female Tg.AC Hemizygous Mice Exposed to Aspartame in Feed for 9 Months

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the lung, salivary gland, testis and epididymis, brain, and adrenal gland. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix A for males and Appendix B for females.

Lung: Alveolar/bronchiolar adenomas were seen in a few 6,250, 12,500, 25,000, and 50,000 ppm males (0 ppm, 0/14; 3,125 ppm, 0/15; 6,250 ppm, 2/14; 12,500 ppm, 1/15; 25,000 ppm, 2/14; 50,000 ppm, 2/14; Table A3).

Salivary Gland: Carcinomas were seen in a few 6,250, 25,000, and 50,000 ppm males (0/15, 0/15, 2/15, 0/15, 1/15, 1/15), and in 12,500 and 50,000 ppm females (0/15, 0/15, 0/15, 1/15, 0/15, and 1/15; Tables A3 and B3). No adenomas were observed in these studies.

Testis and Epididymis: The occurrence of germ cell degeneration of the testis was increased in all exposed groups of males (Tables 5 and A4). The lesion was predominantly unilateral, diffusely affecting the testis with mild to moderate severity, and often accompanied by dilated seminiferous tubules, cystic rete testes, aspermia of the epididymis, and occasional granulomas in the epididymis. The germ cell degeneration was consistent with obstruction atrophy of the testes as described by McIntyre *et al*., (2002). The spermatic outflow tract obstruction can be anywhere downstream of the testes, and there is subsequent pressure atrophy of the testis leading to degeneration and aspermia. The lack of exposure concentration-related response, the predominant finding of unilateral rather than bilateral lesions, and the fact that the findings were limited to the Tg.AC hemizygous strain argue against a relationship to aspartame exposure.

Brain: There were four incidences of nonneoplastic focal lesions: a brain abscess in one 25,000 ppm female, focal cortical neuronal necrosis in one 6,250 ppm female, and focal cortical degeneration in one 50,000 ppm male and one 50,000 ppm female (Tables 5, A4, and B4). The lesions are considered incidental and unrelated to exposure. They may be related to a condition described in the parent FVB/N strain in which mice are susceptible to spontaneous seizures and exhibit secondary neuronal necrosis microscopically (Goelz *et al.,* 1998).

Adrenal Gland: The incidences of focal subcapsular adrenocortical hyperplasia were decreased in all exposed groups of females, and the decreases in 12,500 and 25,000 ppm females were significant (Tables 5 and B4). The incidences of focal hypertrophy of the adrenal cortex were decreased in all exposed groups of males (Tables 5 and A4). The incidences of adrenal cortex atrophy tended to decrease at higher exposure concentrations in males; however, in females the incidences at the higher exposure concentrations were slightly increased when compared with controls. The differences in these lesion incidences were not considered related to aspartame exposure.

TABLE 5
Incidences of Selected Nonneoplastic Lesions in Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame

	0 ppm		3,125 ppm		6,250 ppm		12,500 ppm		25,000 ppm		50,000 ppm	
Male												
Adrenal Cortex[a]	13		15		13		14		15		13	
Atrophy[b]	10	(2.1)[c]	12	(1.6)	8	(1.4)	9	(1.2)	8	(1.5)	6	(1.2)
Hypertrophy, Focal	9	(1.0)	5*	(1.0)	5	(1.0)	6	(1.0)	7	(1.0)	5	(1.0)
Brain	15		15		13		14		14		15	
Cortex, Cerebrum, Degeneration, Focal	0		0		0		0		0		1	(1.0)
Epididymis	15		15		15		15		15		15	
Aspermia, Bilateral	0		1		0		0		0		0	
Aspermia (includes bilateral)	1		4		4		2		3		2	
Testes	15		15		15		14		15		15	
Germinal Epithelium Degeneration, Bilateral	0		2	(3.0)	0		1	(1.0)	0		1	(2.0)
Germinal Epithelium, Degeneration (includes bilateral)	1	(3.0)	4	(3.0)	4	(2.5)	3	(2.0)	3	(2.3)	4	(2.5)
Female												
Adrenal Cortex	15		12		13		13		14		15	
Atrophy	3	(2.0)	2	(2.0)	1	(1.0)	4	(1.0)	5	(1.6)	5	(1.4)
Subcapsular, Hyperplasia, Focal	12	(1.0)	6	(1.0)	6	(1.0)	5*	(1.0)	6*	(1.0)	6	(1.0)
Brain	15		12		13		13		14		11	
Abscess, Focal	0		0		0		0		1	(3.0)	0	
Cortex, Cerebrum, Degeneration, Focal	0		0		0		0		0		1	(1.0)
Cortex, Cerebrum, Neuron, Necrosis	0		0		1	(1.0)	0		0		0	

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

9-Month Study in p53 Haploinsufficient Mice

Survival

Estimates of 9-month survival probabilities for male and female mice are shown in Table 6. Survival of all exposed groups was similar to that of the control groups.

Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of exposed males were generally similar to those of controls through week 28. Mean body weights of 6,250, 12,500, 25,000, and 50,000 ppm males were less than those of the controls for several weeks near the end of the study (Figure 5 and Table 7). Mean body weights of all exposed groups of females were similar to those of the control groups (Figure 5 and Table 8). Feed consumption by the exposed groups was similar to that by the control groups (Tables J3 and J4). Dietary concentrations of 3,125, 6,250, 12,500, 25,000, and 50,000 ppm aspartame delivered average daily doses of approximately 490, 970, 1,860, 3,800, and 7,280 mg/kg to males and 630, 1,210, 2,490, 5,020, and 9,620 mg/kg to females. There were no clinical findings related to aspartame exposure.

Organ Weights and Organ-Weight-to-Body-Weight Ratios

There were no chemical-related differences in absolute or relative organ weights in males or females (Table H2).

Pathology and Statistical Analyses

There were no neoplasms or nonneoplastic lesions in p53 haploinsufficient mice that were attributed to exposure to aspartame. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix C for males and Appendix D for females.

TABLE 6
Survival of p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Male						
Animals initially in study	15	15	15	15	15	15
Moribund	0	0	1	0	0	1
Natural deaths	1	0	1	0	1	0
Animals surviving to study termination	14	15	13	15	14	14
Percent probability of survival at end of study[a]	93	100	87	100	93	93
Mean survival (days)[b]	263	274	259	274	259	261
Survival analysis[c]	P=1.000	P=1.000N	P=1.000	P=1.000N	P=1.000	P=1.000
Female						
Animals initially in study	15	15	15	15	15	15
Moribund	1	0	1	0	0	0
Natural deaths	0	1	0	0	0	0
Animals surviving to study termination	14	14	14	15	15	15
Percent probability of survival at end of study	93	93	93	100	100	100
Mean survival (days)	259	263	268	275	275	275
Survival analysis	P=0.363N	P=1.000N	P=1.000N	P=1.000N	P=1.000N	P=1.000N

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice).
[c] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed group columns. A negative trend or lower mortality in an exposed group is indicated by **N**.

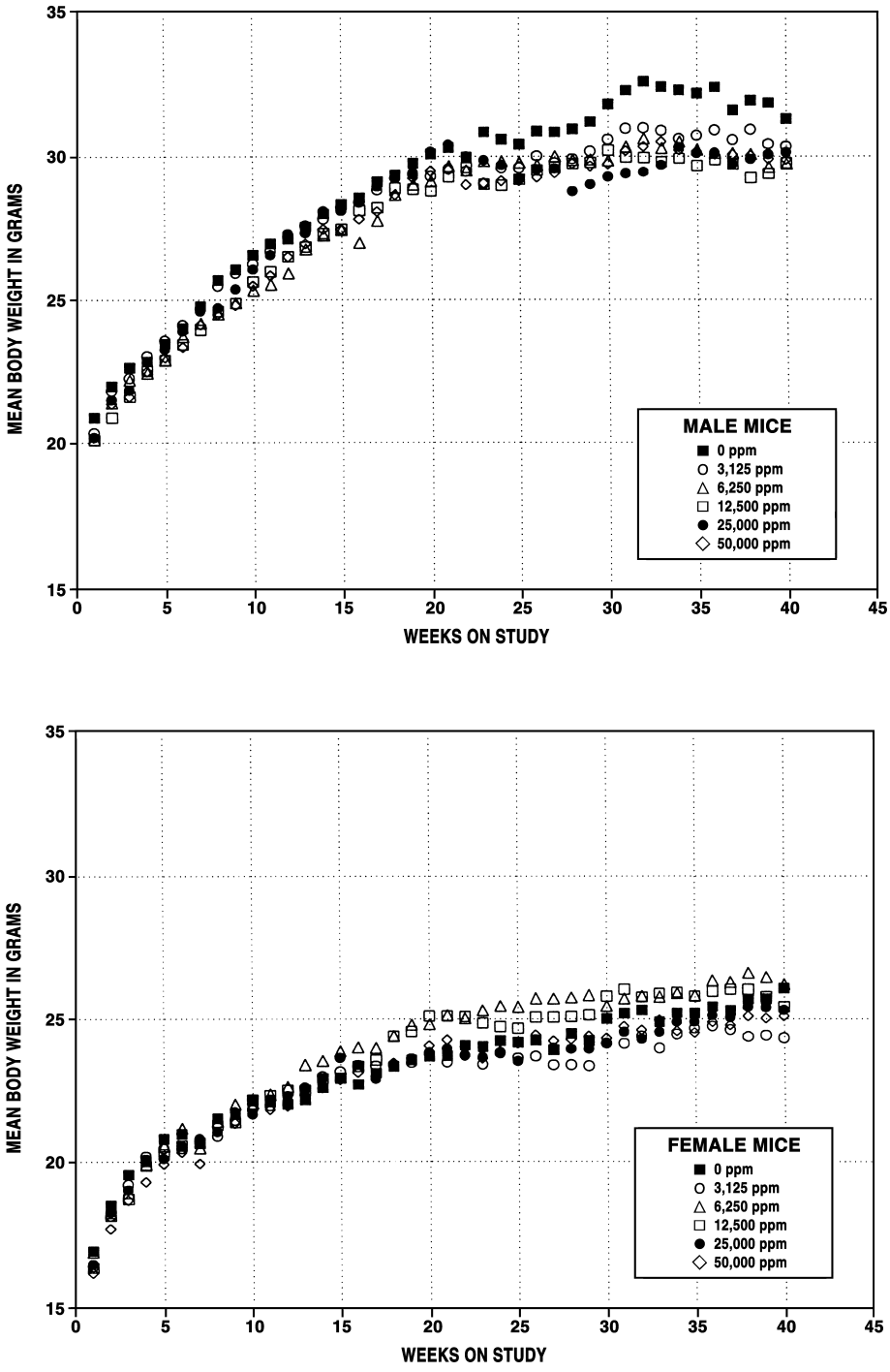


FIGURE 5
Growth Curves for Male and Female p53 Haploinsufficient Mice Exposed to Aspartame in Feed for 9 Months

TABLE 7
Mean Body Weights and Survival of Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame

Weeks on Study	0 ppm		3,125 ppm			6,250 ppm			12,500 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	20.9	15	20.3	97	15	20.1	96	15	20.1	96	15
2	22.0	15	21.8	99	15	21.4	97	15	20.9	95	15
3	22.6	15	22.3	99	15	22.2	98	15	21.6	96	15
4	22.8	15	23.0	101	15	22.4	98	15	22.5	99	15
5	23.5	15	23.6	100	15	22.9	97	15	22.9	97	15
6	24.0	15	24.1	100	15	23.7	99	15	23.4	98	15
7	24.8	15	24.7	100	15	24.2	98	15	23.9	96	15
8	25.7	15	25.5	99	15	24.5	95	15	24.6	96	15
9	26.1	15	25.9	99	15	24.9	95	15	24.9	95	15
10	26.6	15	26.2	99	15	25.3	95	15	25.6	96	15
11	27.0	15	26.7	99	15	25.5	94	15	26.0	96	15
12	27.1	15	27.2	100	15	25.9	96	15	26.5	98	15
13	27.6	15	27.6	100	15	26.8	97	15	26.8	97	15
14	28.0	15	27.8	99	15	27.3	98	15	27.3	98	15
15	28.3	15	28.2	100	15	27.4	97	15	27.5	97	15
16	28.6	14	28.4	99	15	27.0	94	15	28.1	98	15
17	29.1	14	28.8	99	15	27.8	96	15	28.2	97	15
18	29.4	14	29.2	99	15	28.7	98	14	28.9	98	15
19	29.8	14	29.4	99	15	29.0	97	14	28.9	97	15
20	30.1	14	29.3	97	15	29.1	97	14	28.8	96	15
21	30.3	14	29.6	98	15	29.7	98	14	29.3	97	15
22	30.0	14	29.5	98	15	29.5	98	14	29.6	99	15
23	30.8	14	29.0	94	15	29.8	97	14	29.0	94	15
24	30.6	14	29.6	97	15	29.8	97	14	29.0	95	15
25	30.4	14	29.6	97	15	29.8	98	14	29.2	96	15
26	30.9	14	30.0	97	15	29.7	96	14	29.5	96	15
27	30.8	14	29.8	97	15	30.0	97	14	29.6	96	15
28	31.0	14	29.9	97	15	29.9	97	14	29.7	96	15
29	31.2	14	30.2	97	15	29.9	96	14	29.8	96	15
30	31.8	14	30.6	96	15	29.8	94	14	30.2	95	15
31	32.3	14	31.0	96	15	30.3	94	13	30.0	93	15
32	32.6	14	31.0	95	15	30.6	94	13	29.9	92	15
33	32.4	14	30.9	95	15	30.3	94	13	29.8	92	15
34	32.3	14	30.6	95	15	30.5	94	13	29.9	93	15
35	32.2	14	30.7	95	15	30.2	94	13	29.7	92	15
36	32.4	14	30.9	95	15	30.1	93	13	29.9	92	15
37	31.6	14	30.6	97	15	30.1	95	13	29.7	94	15
38	31.9	14	30.9	97	15	30.1	94	13	29.3	92	15
39	31.9	14	30.4	95	15	29.6	93	13	29.4	92	15
40	31.3	14	30.3	97	15	29.7	95	13	29.8	95	15
Mean for weeks											
1-13	24.7		24.5	99		23.8	96		23.8	96	
14-40	30.8		29.9	97		29.5	96		29.3	95	

TABLE 7
Mean Body Weights and Survival of Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame

Weeks on Study	25,000 ppm			50,000 ppm		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	20.2	97	15	20.2	97	15
2	21.5	98	15	21.4	97	15
3	21.8	97	15	21.6	96	15
4	22.8	100	15	22.5	99	15
5	23.3	99	15	23.0	98	15
6	23.9	100	15	23.3	97	15
7	24.6	99	15	24.1	97	15
8	24.7	96	14	24.5	95	15
9	25.4	97	14	24.8	95	15
10	26.1	98	14	25.5	96	15
11	26.6	99	14	25.8	96	14
12	27.3	101	14	26.5	98	14
13	27.3	99	14	27.0	98	14
14	28.1	100	14	27.5	98	14
15	28.1	99	14	27.5	97	14
16	28.4	99	14	27.8	97	14
17	29.0	100	14	28.1	97	14
18	29.3	100	14	28.7	98	14
19	29.4	99	14	29.2	98	14
20	30.2	100	14	29.5	98	14
21	30.4	100	14	29.5	97	14
22	30.0	100	14	29.0	97	14
23	29.9	97	14	29.1	95	14
24	29.7	97	14	29.1	95	14
25	29.2	96	14	29.1	96	14
26	29.5	96	14	29.3	95	14
27	29.6	96	14	29.4	96	14
28	28.8	93	14	29.7	96	14
29	29.0	93	14	29.7	95	14
30	29.3	92	14	29.7	93	14
31	29.4	91	14	30.2	94	14
32	29.5	91	14	30.3	93	14
33	29.7	92	14	30.5	94	14
34	30.3	94	14	30.2	94	14
35	30.1	94	14	30.2	94	14
36	30.1	93	14	30.1	93	14
37	29.7	94	14	30.1	95	14
38	29.9	94	14	30.0	94	14
39	30.1	94	14	29.9	94	14
40	30.1	96	14	29.9	96	14
Mean for weeks						
1-13	24.3	98		23.9	97	
14-40	29.5	96		29.4	95	

TABLE 8
Mean Body Weights and Survival of Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame

Weeks on Study	0 ppm		3,125 ppm			6,250 ppm			12,500 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	16.9	15	16.4	97	15	16.8	99	15	16.3	96	15
2	18.5	15	18.1	98	15	18.2	98	15	18.1	98	15
3	19.6	15	19.2	98	15	18.9	96	15	18.7	95	15
4	20.0	15	20.2	101	15	19.9	100	15	19.9	100	15
5	20.8	15	20.5	99	15	20.5	99	15	20.3	98	15
6	21.0	15	20.5	98	15	21.2	101	15	20.6	98	15
7	20.7	14	20.7	100	15	20.5	99	15	20.6	100	15
8	21.5	14	20.9	97	15	21.4	100	15	21.3	99	15
9	21.7	14	21.5	99	15	22.0	101	15	21.4	99	15
10	22.1	14	21.8	99	15	22.0	100	15	22.2	101	15
11	22.1	14	22.0	100	15	22.4	101	15	22.3	101	15
12	22.0	14	22.0	100	15	22.6	103	15	22.5	102	15
13	22.2	14	22.6	102	15	23.4	105	15	22.5	101	15
14	22.6	14	23.0	102	15	23.5	104	15	22.9	101	15
15	22.9	14	23.2	101	14	23.9	104	15	22.9	100	15
16	22.7	14	23.3	103	14	24.0	106	15	23.3	103	15
17	23.1	14	23.4	101	14	24.0	104	15	23.6	102	15
18	23.3	14	23.4	100	14	24.4	105	15	24.4	105	15
19	23.6	14	23.5	100	14	24.8	105	15	24.6	104	15
20	23.7	14	23.8	100	14	24.8	105	15	25.1	106	15
21	23.7	14	23.5	99	14	25.1	106	15	25.1	106	15
22	24.1	14	23.7	98	14	25.0	104	15	25.1	104	15
23	24.0	14	23.4	98	14	25.3	105	15	24.9	104	15
24	24.2	14	23.8	98	14	25.5	105	15	24.7	102	15
25	24.2	14	23.6	98	14	25.4	105	14	24.7	102	15
26	24.3	14	23.7	98	14	25.7	106	14	25.1	103	15
27	23.9	14	23.4	98	14	25.7	108	14	25.1	105	15
28	24.5	14	23.4	96	14	25.8	105	14	25.1	102	15
29	24.2	14	23.4	97	14	25.8	107	14	25.1	104	15
30	25.0	14	24.1	96	14	25.5	102	14	25.8	103	15
31	25.2	14	24.1	96	14	25.7	102	14	26.0	103	15
32	25.3	14	24.4	96	14	25.8	102	14	25.8	102	15
33	24.9	14	24.0	96	14	25.8	104	14	25.9	104	15
34	25.2	14	24.5	97	14	25.9	103	14	25.9	103	15
35	25.2	14	24.7	98	14	25.8	102	14	25.8	102	15
36	25.4	14	24.7	97	14	26.4	104	14	26.0	102	15
37	25.3	14	24.6	97	14	26.3	104	14	26.0	103	15
38	25.7	14	24.4	95	14	26.6	104	14	26.0	101	15
39	25.7	14	24.4	95	14	26.5	103	14	25.8	100	15
40	26.1	14	24.3	93	14	26.2	100	14	25.4	97	15
Mean for weeks											
1-13	20.7		20.5	99		20.8	100		20.5	99	
14-40	24.4		23.8	98		25.4	104		25.0	102	

TABLE 8
Mean Body Weights and Survival of Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame

Weeks on Study	25,000 ppm Av. Wt. (g)	25,000 ppm Wt. (% of controls)	25,000 ppm No. of Survivors	50,000 ppm Av. Wt. (g)	50,000 ppm Wt. (% of controls)	50,000 ppm No. of Survivors
1	16.4	97	15	16.1	95	15
2	18.2	98	15	17.7	96	15
3	19.0	97	15	18.6	95	15
4	20.1	101	15	19.3	97	15
5	20.1	97	15	19.9	96	15
6	20.6	98	15	20.3	97	15
7	20.8	101	15	19.9	96	15
8	21.0	98	15	21.3	99	15
9	21.7	100	15	21.3	98	15
10	21.7	98	15	21.9	99	15
11	22.2	101	15	21.8	99	15
12	22.3	101	15	21.9	100	15
13	22.5	101	15	22.3	101	15
14	22.9	101	15	22.6	100	15
15	23.6	103	15	22.9	100	15
16	23.4	103	15	23.1	102	15
17	22.9	99	15	23.0	100	15
18	23.3	100	15	23.5	101	15
19	23.6	100	15	23.6	100	15
20	23.8	100	15	24.1	102	15
21	24.0	101	15	24.3	103	15
22	23.7	98	15	24.0	100	15
23	23.7	99	15	23.6	98	15
24	23.8	98	15	24.2	100	15
25	23.5	97	15	24.1	100	15
26	24.3	100	15	24.4	100	15
27	24.0	100	15	24.2	101	15
28	23.9	98	15	24.3	99	15
29	24.0	99	15	24.4	101	15
30	24.2	97	15	24.3	97	15
31	24.5	97	15	24.7	98	15
32	24.3	96	15	24.6	97	15
33	24.5	98	15	25.0	100	15
34	24.9	99	15	24.6	98	15
35	24.9	99	15	24.5	97	15
36	25.1	99	15	24.9	98	15
37	25.0	99	15	24.8	98	15
38	25.4	99	15	25.1	98	15
39	25.4	99	15	25.0	97	15
40	25.3	97	15	25.1	96	15
Mean for weeks						
1-13	20.5	99		20.2	98	
14-40	24.1	99		24.2	99	

9-Month Study in Cdkn2a deficient mice

Estimates of 9-month survival probabilities for male and female mice are shown in Table 9. Survival of all exposed groups was similar to that of the control groups.

Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of 3,125 ppm males after week 29 and of 6,250 ppm males after week 16 were less than those of the controls (Figure 6 and Table 10). Mean body weights of female mice were similar to those of the controls throughout the study (Figure 6 and Table 11). Feed consumption by exposed groups was similar to that by the control groups (Tables J5 and J6). Dietary concentrations of 3,125, 6,250, 12,500, 25,000, and 50,000 ppm aspartame delivered average daily doses of approximately 490, 960, 1,900, 3,700, and 7,400 mg/kg to males and 610, 1,200, 2,300, 4,850, and 9,560 mg/kg to females. There were no clinical findings related to aspartame exposure.

Organ Weights and Organ-Weight-to-Body-Weight Ratios

No statistical differences were found in absolute organ weights, but relative lung weights of 6,250 and 25,000 ppm female mice were significantly decreased (Table H3).

Table 9
Survival of Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Male						
Animals initially in study	15	15	15	15	15	15
Natural deaths	1	1	0	1	1	0
Animals surviving to study termination	14	14	15	14	14	15
Percent probability of survival at end of study[a]	93	93	100	93	93	100
Mean survival (days)[b]	260	271	274	259	257	274
Survival analysis[c]	P=0.741N	P=1.000N	P=1.000N	P=1.000	P=1.000	P=1.000N
Female						
Animals initially in study	15	15	15	15	15	15
Moribund	0	0	0	0	0	1
Natural deaths	2	0	2	0	0	0
Animals surviving to study termination	13	15	13	15	15	14
Percent probability of survival at end of study	87	100	87	100	100	93
Mean survival (days)	254	275	260	275	275	275
Survival analysis	P=0.851N	P=0.464N	P=1.000	P=0.464N	P=0.464N	P=0.951N

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice).
[c] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox 1972) with the controls are in the exposed group columns. A negative trend or lower mortality in an exposed group is indicated by **N**.

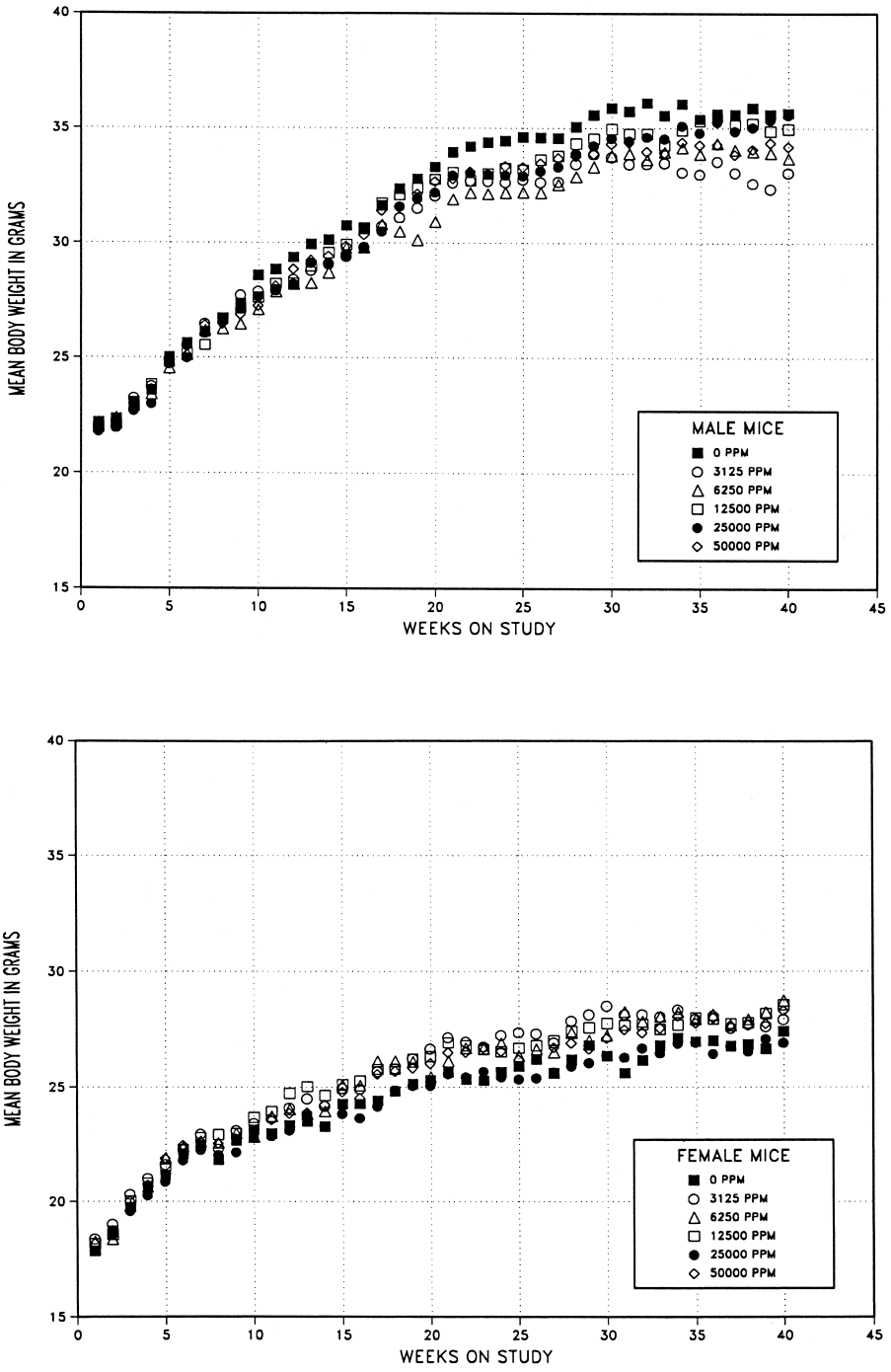


FIGURE 6
Growth Curves for Male and Female Cdkn2a Deficient Mice Exposed to Aspartame in Feed for 9 Months

TABLE 10
Mean Body Weights and Survival of Male Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame

Weeks on Study	0 ppm		3,125 ppm			6,250 ppm			12,500 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	22.2	15	22.1	100	15	22.2	100	15	22.0	99	15
2	22.4	15	22.2	99	15	22.4	100	15	22.1	99	15
3	23.1	15	23.2	100	15	23.0	100	15	22.9	99	15
4	23.6	15	23.8	101	15	23.4	99	15	23.8	101	15
5	25.0	15	24.9	100	15	24.6	98	15	24.8	99	15
6	25.6	15	25.5	100	15	25.1	98	15	25.2	98	15
7	26.1	15	26.5	102	15	26.2	100	15	25.6	98	15
8	26.7	15	26.7	100	15	26.3	99	15	26.7	100	14
9	27.4	15	27.7	101	15	26.5	97	15	27.2	99	14
10	28.6	14	27.9	98	15	27.1	95	15	27.6	97	14
11	28.9	14	27.9	97	15	27.9	97	15	28.2	98	14
12	29.4	14	28.4	97	15	28.2	96	15	28.3	96	14
13	30.0	14	28.8	96	15	28.3	94	15	29.0	97	14
14	30.1	14	29.1	97	15	28.7	95	15	29.6	98	14
15	30.8	14	29.4	96	15	29.8	97	15	29.9	97	14
16	30.6	14	29.8	97	15	29.8	97	15	30.7	100	14
17	31.6	14	30.7	97	15	30.8	98	15	31.7	100	14
18	32.4	14	31.1	96	15	30.5	94	15	32.1	99	14
19	32.8	14	31.5	96	15	30.1	92	15	32.4	99	14
20	33.3	14	32.1	96	15	30.9	93	15	32.8	99	14
21	34.0	14	32.6	96	15	31.9	94	15	33.1	97	14
22	34.2	14	32.7	96	15	32.2	94	15	32.7	96	14
23	34.4	14	32.7	95	15	32.2	94	15	33.0	96	14
24	34.5	14	32.7	95	15	32.2	93	15	33.3	97	14
25	34.6	14	32.8	95	15	32.2	93	15	33.2	96	14
26	34.6	14	32.7	95	15	32.2	93	15	33.6	97	14
27	34.6	14	32.7	95	15	32.6	94	15	33.8	98	14
28	35.1	14	33.4	95	15	32.9	94	15	34.3	98	14
29	35.6	14	33.9	95	15	33.3	94	15	34.6	97	14
30	35.9	14	33.8	94	15	33.8	94	15	35.0	98	14
31	35.8	14	33.4	93	15	33.9	95	15	34.8	97	14
32	36.1	14	33.5	93	15	33.7	93	15	34.8	96	14
33	35.6	14	33.5	94	15	34.0	96	15	34.3	96	14
34	36.1	14	33.1	92	15	34.2	95	15	34.9	97	14
35	35.4	14	33.0	93	14	33.9	96	15	35.3	100	14
36	35.6	14	33.6	94	14	34.3	96	15	35.5	100	14
37	35.6	14	33.1	93	14	34.1	96	15	35.1	99	14
38	35.9	14	32.6	91	14	34.0	95	15	35.2	98	14
39	35.6	14	32.4	91	14	33.9	95	15	34.9	98	14
40	35.7	14	33.1	93	14	33.7	94	15	35.0	98	14
Mean for weeks											
1-13	26.1		25.8	99		25.5	98		25.6	98	
14-40	34.3		32.4	94		32.4	94		33.5	98	

TABLE 10
Mean Body Weights and Survival of Male Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame

Weeks on Study	25,000 ppm Av. Wt. (g)	25,000 ppm Wt. (% of controls)	25,000 ppm No. of Survivors	50,000 ppm Av. Wt. (g)	50,000 ppm Wt. (% of controls)	50,000 ppm No. of Survivors
1	21.8	98	15	21.9	99	15
2	22.0	98	15	22.3	100	15
3	22.7	98	15	23.0	100	15
4	23.0	98	14	23.6	100	15
5	24.7	99	14	24.9	100	15
6	25.0	98	14	25.1	98	15
7	26.1	100	14	26.4	101	15
8	26.6	100	14	26.5	99	15
9	27.1	99	14	26.9	98	15
10	27.7	97	14	27.3	96	15
11	28.0	97	14	28.1	97	15
12	28.2	96	14	28.9	98	15
13	29.1	97	14	29.2	97	15
14	29.1	97	14	29.4	98	15
15	29.5	96	14	29.9	97	15
16	29.8	97	14	30.4	99	15
17	30.5	97	14	31.4	99	15
18	31.6	98	14	31.6	98	15
19	32.0	98	14	32.1	98	15
20	32.2	97	14	32.7	98	15
21	33.0	97	14	32.8	97	15
22	33.1	97	14	33.1	97	15
23	33.0	96	14	33.0	96	15
24	33.0	96	14	33.4	97	15
25	32.9	95	14	33.3	96	15
26	33.1	96	14	33.5	97	15
27	33.3	96	14	33.7	97	15
28	33.9	97	14	33.8	96	15
29	34.3	96	14	33.9	95	15
30	34.6	96	14	34.3	96	15
31	34.5	96	14	34.4	96	15
32	34.6	96	14	34.0	94	15
33	34.6	97	14	33.9	95	15
34	35.1	97	14	34.4	95	15
35	34.8	98	14	34.3	97	15
36	35.3	99	14	34.3	96	15
37	34.9	98	14	33.9	95	15
38	35.0	98	14	34.1	95	15
39	35.4	99	14	34.4	97	15
40	35.6	100	14	34.2	96	15
Mean for weeks						
1-13	25.5	98		25.7	98	
14-40	33.3	97		33.1	97	

TABLE 11
Mean Body Weights and Survival of Female Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame

Weeks on Study	0 ppm		3,125 ppm			6,250 ppm			12,500 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	17.9	15	18.4	103	15	18.3	102	15	18.2	102	15
2	18.7	15	19.0	102	15	18.4	98	15	18.6	100	15
3	19.8	14	20.3	103	15	19.8	100	15	20.0	101	15
4	20.7	14	21.0	101	15	20.6	100	15	20.8	101	15
5	21.2	14	21.6	102	15	21.9	103	15	21.4	101	15
6	22.1	14	22.3	101	15	22.4	101	15	22.3	101	15
7	22.5	14	23.0	102	15	22.6	100	15	22.8	101	15
8	21.8	14	22.3	102	15	22.6	104	15	22.9	105	15
9	22.7	14	23.1	102	15	23.0	101	15	23.0	101	15
10	23.1	14	23.4	101	15	22.8	99	15	23.7	103	15
11	23.0	14	23.6	103	15	23.7	103	15	23.9	104	15
12	23.3	14	24.1	103	15	24.0	103	15	24.7	106	15
13	23.5	14	24.5	104	15	23.8	101	15	25.0	106	15
14	23.3	14	24.2	104	15	24.0	103	15	24.6	106	15
15	24.3	14	25.0	103	15	25.1	103	15	25.1	103	15
16	24.3	14	24.9	103	15	25.1	103	15	25.3	104	15
17	24.4	14	25.7	105	15	26.1	107	14	25.8	106	15
18	24.8	14	25.8	104	15	26.1	105	14	25.8	104	15
19	25.1	14	26.3	105	15	26.1	104	14	26.2	104	15
20	25.3	14	26.7	106	15	25.5	101	14	26.3	104	15
21	25.7	14	27.2	106	15	26.1	102	14	26.9	105	15
22	25.4	14	27.0	106	15	26.7	105	14	26.8	106	15
23	25.3	14	26.8	106	15	26.7	106	14	26.6	105	15
24	25.7	14	27.2	106	15	26.9	105	14	26.5	103	15
25	25.9	14	27.4	106	15	26.4	102	14	26.7	103	15
26	26.2	14	27.3	104	15	26.7	102	14	26.8	102	15
27	25.6	14	26.9	105	15	26.5	104	14	27.0	106	15
28	26.2	14	27.9	107	15	27.4	105	14	27.4	105	15
29	26.8	14	28.1	105	15	27.0	101	14	27.6	103	15
30	26.4	14	28.5	108	15	27.2	103	14	27.8	105	15
31	25.6	14	28.2	110	15	28.3	111	14	27.7	108	15
32	26.2	14	28.1	107	15	27.8	106	13	27.7	106	15
33	26.8	13	28.0	105	15	28.1	105	13	27.5	103	15
34	27.1	13	28.4	105	15	28.3	104	13	27.7	102	15
35	27.0	13	28.0	104	15	27.9	103	13	28.0	104	15
36	27.0	13	28.1	104	15	28.1	104	13	28.0	104	15
37	26.8	13	27.6	103	15	27.7	103	13	27.8	104	15
38	26.9	13	27.7	103	15	28.0	104	13	27.8	103	15
39	26.7	13	27.6	103	15	28.3	106	13	28.2	106	15
40	27.4	13	27.9	102	15	28.8	105	13	28.6	104	15
Mean for weeks											
1-13	21.6		22.0	102		21.8	101		22.1	102	
14-40	25.9		27.1	105		26.9	104		27.0	104	

TABLE 11
Mean Body Weights and Survival of Female Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame

Weeks on Study	25,000 ppm			50,000 ppm		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	17.9	100	15	18.2	102	15
2	18.6	100	15	18.8	101	15
3	19.6	99	15	20.0	101	15
4	20.3	98	15	20.7	100	15
5	20.9	99	15	21.9	103	15
6	21.8	99	15	22.5	102	15
7	22.3	99	15	22.6	100	15
8	22.0	101	15	22.5	103	15
9	22.1	97	15	22.8	100	15
10	22.8	99	15	23.1	100	15
11	22.9	100	15	23.6	103	15
12	23.1	99	15	23.9	103	15
13	23.8	101	15	23.9	102	15
14	23.3	100	15	24.2	104	15
15	23.8	98	15	24.8	102	15
16	23.7	98	15	24.5	101	15
17	24.2	99	15	25.6	105	15
18	24.9	100	15	25.7	104	15
19	25.1	100	15	25.8	103	15
20	25.1	99	15	26.0	103	15
21	25.6	100	15	26.5	103	15
22	25.4	100	15	26.5	104	15
23	25.7	102	15	26.7	106	15
24	25.4	99	15	26.6	104	15
25	25.3	98	15	26.2	101	15
26	25.4	97	15	26.2	100	15
27	25.6	100	15	26.7	104	15
28	25.9	99	15	26.9	103	15
29	26.1	97	15	26.7	100	15
30	26.4	100	15	27.1	103	15
31	26.3	103	15	27.5	107	15
32	26.7	102	15	27.4	105	15
33	26.5	99	15	27.6	103	15
34	26.9	99	15	28.1	104	15
35	26.9	100	15	27.8	103	15
36	26.4	98	15	28.0	104	15
37	26.8	100	15	27.7	103	15
38	26.6	99	15	27.7	103	15
39	27.1	102	15	27.8	104	15
40	26.9	98	15	28.3	103	14
Mean for weeks						
1-13	21.4	99		21.9	102	
14-40	25.7	100		26.7	103	

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions of the liver and kidney. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix E for males and Appendix F for females.

Liver: Minimal to mild cytoplasmic vacuolization of periportal hepatocytes was found in control and exposed male mice (Tables 12 and E4). Compared to the control group, the incidences of periportal vacuolization were significantly greater in males exposed to 6,250, 25,000, or 50,000 ppm aspartame. Severity grades were increased in exposed mice. The prominence of the periportal hepatocellular vacuolization is accentuated by the concurrent loss of vacuolization in the centrilobular hepatocytes (probable glycogenolysis), rather than primarily being due to an accumulation of excessive vacuoles (presumed glycogen) in periportal cells. The biologic significance is uncertain, but this is likely a phenomenon with biological variability reflecting metabolic demands for glycogen and may be related to the amount of time without direct access to food prior to sacrifice rather than to treatment.

Kidney: The incidence of minimal nephropathy in male mice exposed to 50,000 ppm was increased; however, this increase was not significant (Tables 12 and E4). This change consisted of occasional cortical tubules with slight to moderate cytoplasmic basophilia and mild cytomegaly sometimes associated with interstitial inflammatory cell infiltrates, thickening of tubule basement membranes, and proteinaceous tubular casts. Minimal to mild nephropathy can be a common background lesion of uncertain etiology in mice and the biological significance of this marginal increase was not determined.

TABLE 12
Incidences of Selected Nonneoplastic Lesions in Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame

	0 ppm		3,250 ppm		6,250 ppm	12,500 ppm		25,000 ppm	50,000 ppm	
Male										
Liver[a]	15		15		15	14		15	15	
Hepatocyte, Periportal, Vacuolization Cytoplasmic[b]	6	(1.0)[c]	11	(1.5)	14** (1.2)	8	(1.1)	13**(1.1)	13*	(1.5)
Kidney	15		15		15	14		15	15	
Nephropathy	2	(1.0)	0		0	0		0	7	(1.0)
Female										
Liver	15		15		15	15		15	15	
Hepatocyte, Periportal, Vacuolization Cytoplasmic	0		0		0	0		0	2	(1.0)
Kidney	15		15		13	15		15	15	
Nephropathy	1	(1.0)	0		0	0		0	1	(1.0)

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

GENETIC TOXICOLOGY

Aspartame (100 to 10,000 µg/plate) was tested for induction of gene mutations in *Salmonella typhimurium* with and without induced rat or hamster liver S9 metabolic activation enzymes (Table G1). No mutagenicity was detected in strains TA98, TA100, or TA1535 with or without S9. In addition, a single test in TA1537 with 30% rat liver S9 gave negative results. In TA97 with 30% rat liver S9, however, a reproducible small increase in mutant colonies was observed, and this response was judged to be equivocal. No mutagenicity was detected in TA97 without S9 or with hamster liver S9.

No increase in micronucleated polychromatic erythrocytes (PCEs) was observed in bone marrow of male F344/N rats administered aspartame by gavage over a dose range of 500 to 2,000 mg/kg (Table G2). However, a slight decrease in percent PCEs occurred in the 1,000 mg/kg group, and in the positive controls the percent of PCEs was dramatically lowered, an indication of marked toxicity.

Peripheral blood micronucleus tests were conducted in Tg.AC hemizygous, p53 haploinsufficient, and Cdkn2a deficient mice after 9 months exposure to 3,125 to 50,000 ppm aspartame in feed. Negative results were obtained in male and female Tg.AC hemizygous and Cdkn2a deficient mice (Tables G3 and G4). Negative results were also obtained in male p53 haploinsufficient mice (Table G5); in female p53 haploinsufficient mice, the results of the micronucleus test were judged to be positive based on a significant trend test and the increased frequency of micronucleated erythrocytes seen in the 50,000 ppm group. For all three strains of mice, the percent PCEs was not significantly altered by treatment with aspartame.

DISCUSSION AND CONCLUSIONS

Although the National Toxicology Program (NTP) has not conducted standard 2-year rodent bioassays with aspartame, a number of chronic rodent cancer studies have been performed and are outlined in the Introduction to this report. These studies have been interpreted as showing no consistent carcinogenic response that would raise concerns about the safety of this widely consumed artificial sweetener. The one lingering concern has focused on the observation of a low number of brain tumors in studies carried out prior to the registration of the product. Nonetheless, aspartame has been selected for use in the NTP's program for development of alternative carcinogenicity bioassays as a presumed noncarcinogen.

These studies were designed at a time when a number of questions were being raised concerning the adequacy of the existing protocols to provide the maximum sensitivity to detect a positive or carcinogenic response in the genetically modified mouse models employed. These included (1) the belief that the Tg.AC mouse line, while touted as a dermal papilloma model, might also prove to be useful for chemicals administered orally with the "reporter phenotype" (Tennant *et al*., 2001) being papilloma development in the forestomach; (2) the suggestion that studies could be extended to 9 months to increase the opportunity for expression of a positive response in the absence of a significant number of tumors developing in control animals; and (3) the concern that the models in use, i.e. the Tg.AC hemizygous and the p53 haploinsufficient had "blind spots" with regard to the detection of tumors in certain organs such as the brain, prostate gland, liver, and kidney (Bucher, 1998). As a consequence, the current studies were designed to administer aspartame in feed to Tg.AC hemizygous and p53 haploinsufficient mice for 9 months, and similar studies were performed using the Cdkn2a deficient mouse, an entirely uncharacterized model that was believed to hold some promise for detecting cancers of the brain.

There were no prechronic studies performed with these models because prior reports in the literature had shown conclusively that aspartame was well-tolerated by experimental animals given doses of up to 6 to 8 g aspartame/kg body weight per day (Molinary, 1984). In the current studies, the highest dietary concentration of 50,000 ppm was estimated to provide doses of between 6 and 10 g aspartame/kg body weight per day throughout the studies with all three mouse models. There were no specific clinical signs or consistent effects on body weight gains or survival in any of the studies. These findings, coupled with the absence of significant lesions associated with aspartame administration in any of the mouse models studied, suggested that higher doses could have been tolerated by the animals. However, 50,000 ppm has been adopted by the NTP as the maximum exposure concentration in rodent cancer studies with nontoxic substances administered in feed (Bucher, 2000).

In the Tg.AC hemizygous mouse there were a number of neoplasms and nonneoplastic lesions that occurred with slightly different incidences in exposed and control groups. In exposed Tg.AC hemizygous mice, alveolar/bronchiolar adenomas were observed only in males; however, the incidences (up to 2/14 or 14%) were not related to exposure concentration, and these tumors have historically occurred in 4.3% of male control Tg.AC hemizygous mice in 6-month dermal studies (Mahler *et al*., 1998). For these reasons, the alveolar/bronchiolar adenomas were not considered related to aspartame administration. Similarly, there were sporadic incidences of salivary gland carcinomas in exposed groups of male and female Tg.AC hemizygous mice, but again no relation to exposure concentration was apparent. These tumors have also been reported to occur in control Tg.AC mice in 6-month dermal studies, with a higher incidence in homozygous than in hemizygous mice (Tennant *et al*., 2001).

Concerning the typical sites of papilloma formation in Tg.AC hemizygous mice, there were no apparent increases in the incidences of skin or forestomach papillomas in exposed groups compared to the control groups in this study. The rather high incidences of both single

and multiple forestomach papillomas in control Tg.AC hemizygous mice suggest that this model may not be appropriate for use in an extended-duration study of this type, if the chemical is administered orally. The high background papilloma incidence in controls would limit the ability to detect a weak response that lacked a dramatic increase in tumor multiplicity. The lack of a forestomach papilloma response to aspartame was consistent with the expected outcome.

Pritchard *et al*. (2003) reported as part of a global review of transgenic cancer model findings that the Tg.AC model had an overall accuracy of 74% in correctly predicting chemicals that are listed by the International Agency for Research on Cancer and/or the NTP in their respective listings of chemicals as known or suspected human carcinogens. Chemicals studied in the Tg.AC line and found negative also contributed to this accuracy score if the chemicals were considered to be noncarcinogens by virtue of not appearing in these listings. The vast majority of the Tg.AC studies examined by Pritchard *et al*. (2003) used the dermal route of exposure and skin papillomas as the reporter phenotype. The pattern of incorrect assignments included both "false positives" and "false negatives." This level of accuracy is not significantly different from that achieved by the p53 haploinsufficient model or the H*ras*2 model in the Pritchard analysis. However, based on discussions at a recent NTP workshop (NTP, 2003) there is a lack of acceptance within the scientific/regulatory community that a positive result in the Tg.AC model represents a true cancer response. Therefore, the confidence in this model to identify carcinogens appears to be less than that in either the p53 haploinsufficient or H*ras*2 model.

The results of the evaluation of aspartame in the p53 haploinsufficient model were also unremarkable in that no treatment-related neoplasms or nonneoplastic lesions were identified in these mice. The p53 haploinsufficient mouse on the C57BL/6 background has a number of characteristic lesions that typically occur including inflammatory infiltrate in the salivary gland, atrophy of the adrenal cortex, and adrenal subcapsular hyperplasia. These lesions, while usually mild, did occur in this study, but at incidences not affected by aspartame. Although no tumors occurred in control males, 4 of the 15 female control animals developed a neoplasm, a rate higher than in any of the exposed groups. However, no more than one tumor of any particular type occurred in controls, suggesting that the p53 haploinsufficient line maintains sufficiently low background tumor rates to be an effective model if used in a 9-month protocol rather than the typical 6-month study.

The review and analysis of the performance of the p53 haploinsufficient model by Pritchard *et al*. (2003) included data and conclusions as reported by the authors of studies published up to mid-2002. They compared the responses in the p53 haploinsufficient mouse model to a group of 12 known human carcinogens, 19 suspected human carcinogens, and 28 probable human noncarcinogens. In this analysis, the p53 haploinsufficient model had an overall accuracy of 81%. The high accuracy was in large part due to the lack of "false positives" (positive outcomes for probable human noncarcinogens). They concluded that the p53 haploinsufficient model correctly identified 10 of 12 (83%) known human carcinogens, 11 of 19 (58%) suspected carcinogens, and was correctly negative for 27 of 28 (96%) putative noncarcinogens under the conditions of these short-term cancer bioassays. Thus, two known and eight suspected human carcinogens were not detected by the p53 haploinsufficient model. These authors also acknowledged that a number of procedural decisions may have increased the apparent accuracy scores for the mouse models studied. These included such things as accepting a single positive study in a given model as representing a positive response even if other studies of the same chemical were negative or gave equivocal findings in that model.

Clearly, the strength of the p53 haploinsufficient model is in its specificity for correctly identifying known or suspected carcinogens, but its sensitivity to detect carcinogens is a weakness. Because this model either did not identify or only equivocally identified a significant number of known or suspected human carcinogens, negative findings with this model as shown in the current study are of uncertain value.

The p53 haploinsufficient mouse model has been extensively evaluated as an alternative or adjunct assay to the traditional 2-year rodent bioassay (Pritchard *et al*., 2003). In contrast, the Cdkn2a deficient model has seen extremely limited use in this regard, and its ultimate utility as a general chemical cancer screen is uncertain. Inactivation of the p16 gene is seen in a high percentage of human cases of glioma, head and neck squamous cell carcinoma, melanoma, and other cancers (Reed *et al*., 1996; Liggett and Sidransky, 1998). The p16 gene encodes a cell cycle protein that inhibits CDK4 kinases 4 and 6, which in turn prevent phosphorylation of the

Rb protein. This provides a normal control to cell cycle progression from G_1 to S phase. Like the p53 haploinsufficient model, mutations to the p16 gene lead to dysregulation of cell proliferation and should make the mice susceptible to rapid tumor development when exposed to carcinogens. Aspartame had no effect on incidences of neoplasms in this model, but seemed to slightly increase the incidence of mild nephropathy in 50,000 ppm males. Whether this can be ascribed to aspartame or to biological variation is uncertain. The model demonstrated a fairly high background tumor rate for histiocytic sarcoma (2/15 in males and 5/15 in females). Studies of two known rodent carcinogens, phenolphthalein and glycidol, and of the known human carcinogen benzene are underway with the Cdkn2a deficient model, and the results should afford a better comparison of the relative cancer responses of the Cdkn2a deficient and p53 haploinsufficient models.

In summary, there was no evidence of a positive response for papilloma formation in the forestomach or for tumors at other sites in male or female Tg.AC hemizygous mice or for tumors at any site in male or female p53 haploinsufficient mice administered aspartame in feed at concentrations up to 50,000 ppm for 9 months. There was also no evidence of enhanced tumor formation in male or female Cdkn2a deficient mice; this model is currently uncharacterized in terms of its expected response to known rodent and/or human carcinogens and noncarcinogens.

Although there were no increases in neoplasm incidences in any of the mouse models used in these studies, there was a significant increase in the frequency of micronucleated normochromatic erythrocytes at the end of the exposure period in female p53 haploinsufficient mice that received a diet containing 50,000 ppm aspartame. The increase was statistically significant, but the response was small, approximately only one additional micronuclei per 1,000 cells. There was no significant increase in micronucleated erythrocytes in male p53 haploinsufficient mice or in Tg.AC hemizygous or Cdkn2a deficient mice of either sex. In a review of the results of NTP prechronic and chronic studies in which peripheral blood micronuclei had been evaluated in $B6C3F_1$ mice, Witt *et al.* (2000) considered a small increase in magnitude and response and/or a response in only one sex as a weak response, and concluded that weak responses were not well correlated with rodent carcinogenicity and were of uncertain biological significance. By contrast, clearly positive results (determined by the magnitude of the increase in micronucleus frequency and observation of response in both sexes) were associated with high predictivity for rodent carcinogenicity. Because the peripheral blood micronucleus tests are incorporated into the subchronic toxicity assays, they are not repeated and therefore, confirmatory data are not available.

CONCLUSIONS

Under the conditions of this 9-month feed study, there was *no evidence of carcinogenic activity** of aspartame in male or female p53 haploinsufficient mice exposed to 3,125, 6,250, 12,500, 25,000, or 50,000 ppm. Because this is a new model, there is uncertainty whether the study possessed sufficient sensitivity to detect a carcinogenic effect.

---

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Report Review Subcommittee comments and public discussion on this report appears on page 11.

REFERENCES

The Aldrich Library of IR Spectra (1981). 3rd. ed. (C.J. Pouchert, Ed.), Spectrum 1005H. Aldrich Chemical Company, Inc., Milwaukee.

Ashby, J., and Tennant, R.W. (1991). Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP. *Mutat. Res.* **257**, 229-306.

Bailer, A.J., and Portier, C.J. (1988). Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples. *Biometrics* **44**, 417-431.

Bieler, G.S., and Williams, R.L. (1993). Ratio estimates, the delta method, and quantal response tests for increased carcinogenicity. *Biometrics* **49**, 793-801.

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

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

Bradstock, M.K., Serdula, M.K., Marks, J.S., Barnard, R.J., Crane, N.T., Remington, P.L., and Trowbridge, F.L. (1986). Evaluation of reactions to food additives: The aspartame experience. *Am. J. Clin. Nutr.* **43**, 464-469.

Bucher, J.R. (1998). Update on National Toxicology Program (NTP) assays with genetically altered or "transgenic" mice. *Environ. Health Perspect.* **106**, 619-621.

Bucher, J.R. (2000). Doses in rodent cancer studies: Sorting fact from fiction. *Drug Metab. Rev.* **32**, 153-163.

Butchko, H.H., and Stargel, W.W. (2001). Aspartame: Scientific evaluation in the postmarketing period. *Regul. Toxicol. Pharmacol.* **34**, 221-233.

Cannon, R.E., Spalding, J.W., Trempus, C.S., Szczesniak, C.J., Virgil, K.M., Humble, M.C., and Tennant, R.W. (1997). Kinetics of wound-induced v-Ha-ras transgene expression and papilloma development in transgenic Tg.AC mice. *Mol. Carcinog.* **20**, 108-114.

Centers for Disease Control (CDC) (1984). Evaluation of consumer complaints related to aspartame use. *MMWR Morb. Mortal. Wkly. Rep.* **33**, 605-607.

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

Cornell, R.G., Wolfe, R.A, and Sanders, P.G. (1984). Aspartame and brain tumors: Statistical issues. In *Aspartame: Physiology and Biochemistry* (L.D. Stegink and L.J. Filer, Jr., Eds.), pp. 459-479. Marcel Dekker, Inc., New York.

Cox, D.R. (1972). Regression models and life-tables. *J. R. Stat. Soc.* **B34**, 187-220.

Crawford, B.D. (1985). Perspectives on the somatic mutation model of carcinogenesis. In *Advances in Modern Environmental Toxicology. Mechanisms and Toxicity of Chemical Carcinogens and Mutagens* (M.A. Mehlman, W.G. Flamm, and R.J. Lorentzen, Eds.), pp. 13-59. Princeton Scientific Publishing Co., Inc., Princeton, NJ.

Dews, P.B. (1987). Summary report of an international aspartame workshop. *Food Chem. Toxicol.* **25**, 549-552.

Diomede, L., Romano, M., Guiso, G., Caccia, S., Nava, S., and Salmona, M. (1991). Interspecies and interstrain studies on the increased susceptibility to metrazol-induced convulsions in animals given aspartame. *Food Chem. Toxicol.* **29**, 101-106.

Donehower, L.A., Harvey, M., Slagle, B.L., McArthur, M.J., Montogmery, C.A., Jr., Butel, J.S., and Bradley, A. (1992). Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* **356**, 215-221.

Dow-Edwards, D.L., Scribani, L.A., and Riley, E.P. (1989). Impaired performance on odor-aversion testing following prenatal aspartame exposure in the guinea pig. *Neurotoxicol. Teratol.* **11**, 413-416.

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

Federal Register (1981a). Aspartame: Commissioner's Final Decision. Vol. 46, No. 142, pp. 38,285-38,308. Food and Drug Administration, Rockville, MD.

Federal Register (1981b). Aspartame: Commissioner's Final Decision; Correction. Vol. 46, No. 181, p. 46,394. Food and Drug Administration, Rockville, MD.

Federal Register (1984). Food Additives Permitted for Direct Addition to Food for Human Consumption; Aspartame; Denial of Requests for Hearing; Final Rule. Vol. 49, No. 36, pp. 6672-6682. Food and Drug Administration, Department of Health and Human Services, Rockville, MD.

Federal Register (1996). Food Additives Permitted for Direct Addition to Food for Human Consumption; Aspartame. Vol. 61, No. 126, pp. 33,654-33,656. Food and Drug Administration, Department of Health and Human Services, Rockville, MD.

Food and Drug Administration (FDA) (1994). Is aspartame safe? Excerpted from Food allergies are rare but risky. *FDA Consum.*, May 1994 (http:www.cfsan.fda.gov/~dms/qa-adf9.html).

Freeman, G., Sobotka, T., and Hattan, D. (1990). Acute effects of aspartame on concentrations of brain amines and their metabolites in selected brain regions of Fischer 344 and Sprague-Dawley rats. *Drug Chem. Toxicol.* **13**, 113-133.

Galletti, G.C., Chiavari, G., and Bocchini, P. (1995). Thermal decomposition products of aspartame as determined by pyrolysis — gas chromatography/mass spectrometry. *J. Anal. Appl. Pyrolysis* **32**, 137-151.

Goelz, M.F., Mahler, J., Harry, J., Myers, P., Clark, J., Thigpen, J.E., and Forsythe, D.B. (1998). Neuropathologic findings associated with seizures in FVB mice. *Lab. Anim. Sci.* **48**, 34-37.

Gurney, J.G., Pogoda, J.M., Holly, E.A., Hecht, S.S., and Preston-Martin, S. (1997). Aspartame consumption in relation to childhood brain tumor risk: Results from a case-control study. *J. Natl. Cancer Inst.* **89**, 1072-1074.

Harris, C.C. (1996a). Structure and function of the p53 tumor suppressor gene: Clues for rational cancer therapeutic strategies. *J. Natl. Cancer Inst.* **88**, 1442-1455.

Harris, C.C. (1996b). *p53* Tumor suppressor gene: From the basic research laboratory to the clinic — an abridged historical perspective. *Carcinogenesis* **17**, 1187-1198.

Harris, C.C. (1996c). The 1995 Walter Hubert Lecture — molecular epidemiology of human cancer: Insights from the mutational analysis of the p53 tumour-suppressor gene. *Brit. J. Cancer* **73**, 261-269.

Hazardous Substances Data Bank (HSDB) (2003). National Institute for Occupational Safety and Health, HSDB database available through the National Library of Medicine TOXNET System.

Holder, M.D. (1989). Effects of perinatal exposure to aspartame on rat pups. *Neurotoxicol. Teratol.* **11**, 1-6.

Holder, M.D., and Yirmiya, R. (1989). Behavioral assessment of the toxicity of aspartame. *Pharmacol. Biochem. Behav.* **32**, 17-26.

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

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

Ishii, H. (1981). Incidence of brain tumors in rats fed aspartame. *Toxicol. Lett.* **7**, 433-437.

Ishii, H., Koshimizu, T., Usami, S., and Fujimoto, T. (1981). Toxicity of aspartame and its diketopiperazine for Wistar rats by dietary administration for 104 weeks. *Toxicology* **21**, 91-94.

Jeffrey, A.M., and Williams, G.M. (2000). Lack of DNA-damaging activity of five non-nutritive sweeteners in the rat hepatocyte/DNA repair assay. *Food Chem. Toxicol.* **38**, 335-338.

Kaplan, E.L., and Meier, P. (1958). Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.* **53**, 457-481.

Kim, S.K., Jung, M.Y., and Kim, S.Y. (1997). Photodecomposition of aspartame in aqueous solutions. *Food Chem.* **59**, 272-278.

Koestner, A. (1984). Aspartame and brain tumors: Pathology issues. In *Aspartame: Physiology and Biochemistry* (L.D. Stegink and L.J. Filer, Jr., Eds.), pp. 447-457 Marcel Dekker, Inc., New York.

Leder, A., Kuo, A., Cardiff, R.D., Sinn, E., and Leder, P. (1990). v-Ha-*ras* transgene abrogates the initiation step in mouse skin tumorigenesis: Effects of phorbol esters and retinoic acid. *Proc. Natl. Acad. Sci.* **87**, 9178-9182.

Levy, P.S., and Hedeker, D. (1996). Letter to the editor. *J. Neuropathol. Exp. Neurol.* **55**, 1280.

Liggett, W.H., Jr., and Sidransky, D. (1998). Role of the *p16* tumor suppressor gene in cancer. *J. Clin. Oncol.* **16**, 1197-1206.

McAnulty, P.A., Collier, M.J., Enticott, J., Tesh, J.M., Mayhew, D.A., Comer, C.P., Hjelle, J.J., and Kotsonis, F.N. (1989). Absence of developmental effects in CF-1 mice exposed to aspartame *in utero*. *Fundam. Appl. Toxicol.* **13**, 296-302.

MacGregor, J.T., Wehr, C.M., Henika, P.R., and Shelby, M.D. (1990). The *in vivo* erythrocyte micronucleus test: Measurement at steady state increases assay efficiency and permits integration with toxicity studies. *Fundam. Appl. Toxicol.* **14**, 513-522.

McIntyre, B.S., Barlow, N.J., and Foster, P.M.D. (2002). Male rats exposed to linuron *in utero* exhibit perminant changes in anogenital distance, nipple retention, and epididymal malformations that result in subsequent testicular atrophy. *Toxicol Sci.* **65**, 62-70.

Mahler, J.F., Stokes, W., Mann, P.C., Takaoka, M., and Maronpot, R.R. (1996). Spontaneous lesions in aging FVB/N mice. *Toxicol. Pathol.* **24**, 710-716.

Mahler, J.F., Flagler, N.D., Malarkey, D.E., Mann, P.C., Haseman, J.K., and Eastin, W. (1998). Spontaneous and chemically induced proliferative lesions in Tg.AC transgenic and *p53*-heterozygous mice. *Toxicol. Pathol.* **26**, 501-511.

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

Miller, J.A., and Miller, E.C. (1977). Ultimate chemical carcinogens as reactive mutagenic electrophiles. In *Origins of Human Cancer* (H.H. Hiatt, J.D. Watson, and J.A. Winsten, Eds.), pp. 605-627. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Molinary, S.V. (1984). Preclinical studies of aspartame in nonprimate animals. In *Aspartame: Physiology and Biochemistry* (L.D. Stegink and L.J. Filer, Jr., Eds.), pp. 289-306. Marcel Dekker, Inc., New York.

Mukhopadhyay, M., Mukherjee, A., and Chakrabarti, J. (2000). *In vivo* cytogenetic studies on blends of aspartame and acesulfame-K. *Food Chem. Toxicol.* **38**, 75-77.

National Toxicology Program (NTP) (2003). NTP Workshop. Genetically Modified Rodent Models for Cancer Hazard Indentification: Selecting Substances for Study and Interpreting and Communicating Results (http://ntp-server.niehs.nih.gov/Meetings/2003/2003FebTgWkshop.html).

National Toxicology Program (NTP) (2005). Toxicology Studies of Acesulfame Potassium (CAS No. 55589-62-3) in FVB Tg.AC Hemizygous Mice and Carcinogenicity Studies of Acesulfame Potassium in B6/129 (N5) p53 Haploinsufficient Mice (Feed Studies). Technical Report Series No. 524. NIH Publication No. 06-4460. U.S. Department of Health and Human Services, Public Health Service, National Insititutes of Health, Research Triangle Park, NC (in press).

Nguyen, U.N., Dumoulin, G., Henriet, M.-T., and Regnard, J. (1998). Aspartame ingestion increases urinary calcium, but not oxalate excretion, in healthy subjects. *J. Clin. Endocrinol. Metab.* **83**, 165-168.

Olney, J.W., Farber, N.B., Spitznagel, E., and Robins, L.N. (1996). Increasing brain tumor rates: Is there a link to aspartame? *J. Neuropathol. Exp. Neurol.* **55**, 1115-1123.

Piegorsch, W.W., and Bailer, A.J. (1997). *Statistics for Environmental Biology and Toxicology,* Section 6.3.2. Chapman and Hall, London.

Pomerantz, J., Schreiber-Agus, N., Liégeois, N.J., Silverman, A., Alland, L., Chin, L., Potes, J., Chen, K., Orlow, I., Lee, H.-W., Cordon-Cardo, C., and DePinho, R.A. (1998). The *Ink4a* tumor suppressor gene product, $p19^{Arf}$, interacts with MDM2 and neutralizes MDM2's inhibition of p53. *Cell* **92**, 713-723.

Portier, C.J., and Bailer, A.J. (1989). Testing for increased carcinogenicity using a survival-adjusted quantal response test. *Fundam. Appl. Toxicol.* **12,** 731-737.

Portier, C.J., Hedges, J.C., and Hoel, D.G. (1986). Age-specific models of mortality and tumor onset for historical control animals in the National Toxicology Program's carcinogenicity experiments. *Cancer Res.* **46**, 4372-4378.

Pritchard, J.B., French, J.E., Davis, B.J., and Haseman, J.K. (2003). The role of transgenic mouse models in carcinogen identification. *Environ. Health Perspect.* **111**, 444-454.

Ranney, R.E., and Oppermann, J.A. (1979). A review of the metabolism of the aspartyl moiety of aspartame in experimental animals and man. *J. Environ. Pathol. Toxicol.* **2**, 979-985.

Ranney, R.E., Mares, S.E., Schroeder, R.E., Hutsell, T.C., and Radzialowski, F.M. (1975). The phenylalanine and tyrosine content of maternal and fetal body fluids from rabbits fed aspartame. *Toxicol. Appl. Pharmacol.* **32**, 339-346.

Ranney, R.E., Oppermann, J.A., Muldoon, E., and McMahon, F.G. (1976). Comparative metabolism of aspartame in experimental animals and humans. *J. Toxicol. Environ. Health* **2**, 441-451.

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

Rao, G.N., Piegorsch, W.W., Crawford, D.D., Edmondson, J., and Haseman, J.K. (1989b). Influence of viral infections on body weight, survival, and tumor prevalence of $B6C3F_1$ (C57BL/6N × C3H/HeN) mice in carcinogenicity studies. *Fundam. Appl. Toxicol*. **13**, 156-164.

Reed, A.L., Califano, J., Cairns, P., Westra, W.H., Jones, M., Koch, W., Ahrendt, S., Eby, Y., Sewell, D., Nawroz, H., Bartek, J., and Sidransky, D. (1996). High frequency of *p16 (CDKN2/MTS-1/INK4A)* inactivation in head and neck squamous cell carcinoma. *Cancer Res.* **56**, 3630-3633.

Registry of Toxic Effects of Chemical Substances (RTECS) [database online] (2002). Bethesda (MD): National Institute for Occupational Safety and Health; 1971 to present. Updated quarterly. Available from the National Library of Medicine, Bethesda, MD.

Reynolds, W.A., Stegink, L.D., Filer, L.J., Jr., and Renn, E. (1980). Aspartame administration to the infant monkey: Hypothalamic morphology and plasma amino acid levels. *Anat. Rec.* **198**, 73-85.

Reynolds, W.A., Bauman, A.F., Stegink, L.D., and Filer, L.J., Jr. (1984). Developmental assessment of infant macaques receiving dietary aspartame or phenylalanine. In *Aspartame: Physiology and Biochemistry* (L.D. Stegink and L.J. Filer, Jr., Eds.), pp. 405-423. Marcel Dekker, Inc., New York.

Rowan, A.J., Shaywitz, B.A., Tuchman, L., French, J.A., Luciano, D., and Sullivan, C.M. (1995). Aspartame and seizure susceptibility: Results of a clinical study in reportedly sensitive individuals. *Epilepsia* **36**, 270-275.

Serrano, M., Hannon, G.J., and Beach, D. (1993). A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. *Nature* **366**, 704-707.

Serrano, M., Lee, H.-W., Chin, L., Cordon-Cardo, C., Beach, D., and DePinho, R.A. (1996). Role of the *INK4a* locus in tumor suppression and cell mortality. *Cell* **85**, 27-37.

Shelby, M.D. (1988). The genetic toxicity of human carcinogens and its implications. *Mutat. Res.* **204**, 3-15.

Shelby, M.D., and Witt, K.L. (1995). Comparison of results from mouse bone marrow chromosome aberration and micronucleus tests. *Environ. Mol. Mutagen.* **25**, 302-313.

Shelby, M.D., and Zeiger, E. (1990). Activity of human carcinogens in the Salmonella and rodent bone-marrow cytogenetics tests. *Mutat. Res.* **234**, 257-261.

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

Shephard, S.E., Wakabayashi, K., and Nagao, M. (1993). Mutagenic activity of peptides and the artificial sweetener aspartame after nitrosation. *Food Chem. Toxicol.* **31**, 323-329.

Smith, M.A., Freidlin, B., Ries, L.A., and Simon, R. (1998). Trends in reported incidence of primary malignant brain tumors in children in the United States. *J. Natl. Cancer Inst.* **90**, 1269-1277.

Spalding, J.W., Momma, J., Elwell, M.R., and Tennant, R.W. (1993). Chemically induced skin carcinogenesis in a transgenic mouse line (TG•AC) carrying a v-Ha-*ras* gene. *Carcinogenesis* **14**, 1335-1341.

Spalding, J.W., French, J.E., Tice, R.R., Furedi-Machacek, M., Haseman, J.K., and Tennant, R.W. (1999). Development of a transgenic mouse model for carcinogenesis bioassays: Evaluation of chemically induced skin tumors in Tg.AC mice. *Toxicol. Sci.* **49**, 241-254.

Speirs, P.A., Sabounjian, L., Reiner, A., Myers, D.K., Wurtman, J., Schomer, D.L. (1998). Aspartame: Neuropsychologic and nuerophysiologic evaluation of acute and chronic effects. *Am. J. Clin. Nutr.* **68**, 531-537.

Stegink, L.D. (1987). The aspartame story: A model for the clinical testing of a food additive. *Am. J. Clin. Nutr.* **46**, 204-215.

Stegink, L.D., and Filer, L.J., Jr., Eds. (1984). *Aspartame: Physiology and Biochemistry*. Marcel Dekker, Inc., New York.

Stegink, L.D., Filer, L.J., Jr., Baker, G.L., and McDonnell, J.E. (1980). Effect of an abuse dose of aspartame upon plasma and erythrocyte levels of amino acids in phenylketonuric heterozygous and normal adults. *J. Nutr.* **110**, 2216-2224.

Straus, D.S. (1981). Somatic mutation, cellular differentiation, and cancer causation. *JNCI* **67**, 233-241.

Suomi, S.J. (1984). Effects of aspartame on the learning test performance of young stump-tail macaques. In *Aspartame: Physiology and Biochemistry* (L.D. Stegink and L.J. Filer, Jr., Eds.). Marcel Dekker, Inc., New York.

Tarone, R.E. (1975). Tests for trend in life table analysis. *Biometrika* **62**, 679-682.

Tennant, R.W., Margolin, B.H., Shelby, M.D., Zeiger, E., Haseman, J.K., Spalding, J., Caspary, W., Resnick, M., Stasiewicz, S., Anderson, B., and Minor, R. (1987). Prediction of chemical carcinogenicity in rodents from in vitro genetic toxicity assays. *Science* **236**, 933-941.

Tennant, R.W., French, J.E., and Spalding, J.W. (1995). Identifying chemical carcinogens and assessing potential risk in short-term bioassays using transgenic mouse models. *Environ. Health Perspect.* **103**, 942-950.

Tennant, R.W., Spalding, J., and French, J.E. (1996). Evaluation of transgenic mouse bioassays for identifying carcinogens and noncarcinogens. *Mutat. Res.* **365**, 119-127.

Tennant, R.W., Stasiewicz, S., Mennear, J., French, J.E., and Spalding, J.W. (1999). Genetically altered mouse models for identifying carcinogens. *IARC Sci. Publ.* **146**, 123-150.

Tennant, R.W., Stasiewicz, S., Eastin, W.C., Mennear, H., and Spalding, J.W. (2001). The Tg.AC (v-Ha-ras) transgenic mouse: Nature of the model. *Toxicol. Pathol.* **29**, 51-59.

Tilson, H.A., Thai, L., Zhao, D., Sobotka, T.J., and Hong, J.S. (1989). Oral administration of aspartame is not proconvulsant in rats. *Neurotoxicology* **10**, 229-238.

Tilson, H.A., Hong, J.S., and Sobotka, T.J. (1991). High doses of aspartame have no effects on sensorimotor function or learning and memory in rats. *Neurotoxicol. Teratol.* **43**, 27-35.

Tollefson, L. (1988). Monitoring adverse reactions to food additives in the U.S. Food and Drug Administration. *Regul. Toxicol. Pharmacol.* **8**, 438-446.

Tollefson, L., and Barnard, R.J. (1992). An analysis of FDA passive surveillance reports of seizures associated with consumption of aspartame. *J. Am. Diet. Assoc.* **92**, 598-601.

Tomatis, L., Huff, J., Hertz-Picciotto, I., Sandler, D.P., Bucher, J., Boffetta, P., Axelson, O., Blair, A., Taylor, J., Stayner, L., and Barrett, J.C. (1997). Avoided and avoidable risks of cancer. *Carcinogenesis* **18**, 97-105.

Trempus, C.S., Mahler, J.F., Ananthaswamy, H.N., Loughlin, S.M., French, J.E., and Tennant, R.W. (1998). Photocarcinogenesis and susceptibility to UV radiation in the v-Ha-ras transgenic Tg.AC mouse. *J. Invest. Dermatol.* **111**, 445-451.

Trocho, C., Pardo, R., Rafecas, I., Virgili, J., Remesar, X., Fernández-López, J.A., and Alemany, M. (1998). Formaldehyde derived from dietary aspartame binds to tissue components *in vivo.* *Life Sci.* **63**, 337-349.

Van Den Eeden, S.K., Koepsell, T.D., Longstreth, W.T., Jr., van Belle, G., Daling, J.R., and McKnight, B. (1994). Aspartame ingestion and headaches: A randomized crossover trial. *Neurology* **44**, 1787-1793.

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

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

Witt, K.L., Knapton, A., Wehr, C.M., Hook, G.J., Mirsalis, J., Shelby, M.D., and MacGregor, J.T. (2000). Micronucleated erythrocyte frequency in peripheral blood of B6C3F$_1$ mice from short-term, prechronic, and chronic studies of the NTP Carcinogenesis Bioassay Program. *Environ. Mol. Mutagen.* **36**, 163-194.

Wolraich, M.L., Lindgren, S.D., Stumbo, P.J., Stegink, L.D., Appelbaum, M.I., and Kiritsy, M.C. (1994). Effects of diets high in sucrose or aspartame on the behavior and cognitive performance of children. *N. Engl. J. Med.* **330**, 301-307.

Wright, J.T., Hansen, L., Mahler, J., Szczesniak, C., and Spalding, J.W. (1995). Odontogenic tumours in the v-Ha-*ras* (TG•AC) transgenic mouse. *Arch. Oral Biol.* **40**, 631-638.

Zeiger, E., Haseman, J.K., Shelby, M.D., Margolin, B.H., and Tennant, R.W. (1990). Evaluation of four in vitro genetic toxicity tests for predicting rodent carcinogenicity: Confirmation of earlier results with 41 additional chemicals. *Environ. Mol. Mutagen.* **16** (Suppl. 18), 1-14.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K. (1992). Salmonella mutagenicity tests: V Results from the testing of 311 chemicals. *Environ. Mol. Mutagen.* **19** (Suppl. 21), 2-141.

APPENDIX A
SUMMARY OF LESIONS
IN MALE Tg.AC HEMIZYGOUS MICE
IN THE 9-MONTH FEED STUDY
OF ASPARTAME

TABLE A1
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame[a]

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Disposition Summary						
Animals initially in study	15	15	15	15	15	15
Early deaths						
Moribund	3	3	4	2	3	4
Natural deaths	3		3	1	1	1
Survivors						
Terminal sacrifice	9	12	8	12	11	10
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Liver	(15)	(15)	(14)	(15)	(14)	(15)
Salivary glands	(13)		(2)		(2)	(14)
Duct, carcinoma			2 (100%)		1 (50%)	1 (7%)
Stomach, forestomach	(12)	(15)	(13)	(14)	(14)	(14)
Squamous cell papilloma	4 (33%)	1 (7%)	1 (8%)	3 (21%)	4 (29%)	5 (36%)
Squamous cell papilloma, multiple	4 (33%)	4 (27%)	1 (8%)	2 (14%)	2 (14%)	1 (7%)
Tooth	(4)	(4)	(2)	(2)	(2)	(6)
Odontogenic tumor	3 (75%)	4 (100%)	2 (100%)	2 (100%)	2 (100%)	6 (100%)
Cardiovascular System						
None						
Endocrine System						
Adrenal cortex	(13)	(15)	(13)	(14)	(15)	(13)
General Body System						
None						
Genital System						
None						
Hematopoietic System						
Lymph node, mandibular	(12)	(15)	(13)	(14)	(14)	(14)
Lymph node, mesenteric	(13)	(15)	(13)	(14)	(15)	(14)
Lymph node, mediastinal	(11)	(13)	(11)	(10)	(14)	(13)
Spleen	(14)	(15)	(13)	(15)	(14)	(14)
Thymus	(12)	(14)	(12)	(13)	(14)	(12)
Integumentary System						
Skin	(15)	(2)			(1)	(15)
Squamous cell papilloma		2 (100%)			1 (100%)	
Musculoskeletal System						
None						
Nervous System						
None						

TABLE A1
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Respiratory System						
Lung	(14)	(15)	(14)	(15)	(14)	(14)
Alveolar/bronchiolar adenoma			2 (14%)	1 (7%)	2 (14%)	2 (14%)
Special Senses System						
Harderian gland	(15)					(15)
Adenoma						1 (7%)
Zymbal's gland	(14)				(1)	(14)
Carcinoma					1 (100%)	
Urinary System						
Kidney	(15)	(15)	(13)	(15)	(14)	(15)
Systemic Lesions						
Multiple organs[b]	(15)	(15)	(15)	(15)	(15)	(15)
Leukemia erythrocytic	1 (7%)		1 (7%)	2 (13%)		
Lymphoma malignant			1 (7%)			1 (7%)
Neoplasm Summary						
Total animals with primary neoplasms[c]	10	9	7	8	11	11
Total primary neoplasms	12	11	10	10	13	17
Total animals with benign neoplasms	8	6	3	5	8	8
Total benign neoplasms	8	7	4	6	9	9
Total animals with malignant neoplasms	1		4	2	2	2
Total malignant neoplasms	1		4	2	2	2
Total animals with uncertain neoplasms-benign or malignant	3	4	2	2	2	6
Total uncertain neoplasms	3	4	2	2	2	6

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

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame: 0 ppm

Number of Days on Study	0 7 6	1 8 3	1 9 7	2 7 1	2 7 1	2 7 1	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	
Carcass ID Number	0 0 4	0 1 4	0 1 5	0 0 6	0 1 2	0 1 3	0 0 1	0 0 2	0 0 3	0 0 5	0 0 7	0 0 8	0 0 9	0 1 0	0 1 1	Total Tissues/ Tumors
Alimentary System																
Esophagus	+	A	+	+	+	+	+	+	+	+	+	+	+	M	+	13
Gallbladder	A	A	A	+	+	+	+	+	+	M	+	+	M	+	+	10
Intestine large, colon	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	12
Intestine large, rectum	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	12
Intestine large, cecum	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	12
Intestine small, duodenum	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	12
Intestine small, jejunum	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	12
Intestine small, ileum	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	12
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pancreas	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	13
Salivary glands	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	13
Stomach, forestomach	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	12
Squamous cell papilloma							X					X	X	X		4
Squamous cell papilloma, multiple				X	X	X		X								4
Stomach, glandular	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	12
Tongue	+	A	A	+	+	M	+	+	+	+	+	+	+	+	+	12
Tooth	+			+	+	+										4
Odontogenic tumor	X				X	X										3
Cardiovascular System																
Blood vessel	+	A	A	+	+	+	+	+	+	+	+	+	+	M	+	12
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	13
Adrenal medulla	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	13
Islets, pancreatic	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	13
Parathyroid gland	+	A	A	+	M	M	+	M	+	M	I	M	M	+	M	5
Pituitary gland	I	A	A	+	+	+	+	+	+	+	+	+	I	I	I	9
Thyroid gland	A	M	+	+	+	+	+	+	+	+	+	+	+	+	+	13
General Body System																
None																
Genital System																
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Preputial gland	+	A	A	+	+	+	+	+	+	+	+	M	+	+	+	12
Prostate	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	13
Seminal vesicle	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	13
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

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

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous in the 9-Month Feed Study of Aspartame: 0 ppm

Number of Days on Study	0 7 6	1 8 3	1 9 7	2 7 1	2 7 1	2 7 1	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	
Carcass ID Number	0 0 4	0 1 4	0 1 5	0 0 6	0 1 2	0 1 3	0 0 1	0 0 2	0 0 3	0 0 5	0 0 7	0 0 8	0 0 9	0 1 0	0 1 1	Total Tissues/ Tumors
Hematopoietic System																
Bone marrow	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mandibular	+	A	M	+	+	+	+	+	+	M	+	+	+	+	+	12
Lymph node, mesenteric	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	13
Lymph node, mediastinal	+	M	A	+	I	I	+	+	+	+	+	+	+	+	+	11
Spleen	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Thymus	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	12
Integumentary System																
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Musculoskeletal System																
Bone	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	13
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Nervous System																
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Respiratory System																
Larynx	I	A	A	I	M	I	+	I	I	I	+	I	+	M	M	3
Lung	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Nose	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Trachea	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	13
Special Senses System																
Eye	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	12
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Zymbal's gland	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Urinary bladder	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	13
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Leukemia erythrocytic			X													1

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame: 3,125 ppm

Number of Days on Study	1 9 8	2 7 1	2 7 1	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	
Carcass ID Number	0 2 9	0 2 4	0 2 5	0 1 6	0 1 7	0 1 8	0 1 9	0 2 0	0 2 1	0 2 2	0 2 3	0 2 6	0 2 7	0 2 8	0 3 0	Total Tissues/ Tumors
Alimentary System																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Squamous cell papilloma												X				1
Squamous cell papilloma, multiple							X	X		X					X	4
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Tooth	+	+	+					+								4
Odontogenic tumor	X	X	X					X								4
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pituitary gland	+	+	I	+	+	+	M	+	+	+	+	+	+	+	+	13
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
None																
Genital System																
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Penis	+															1
Preputial gland												+				1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mediastinal	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	13
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Integumentary System																
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Skin									+						+	2
Squamous cell papilloma									X						X	2
Musculoskeletal System																
None																
Nervous System																
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame: 3,125 ppm

Number of Days on Study	1 9 8	2 7 1	2 7 1	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	
Carcass ID Number	0 2 9	0 2 4	0 2 5	0 1 6	0 1 7	0 1 8	0 1 9	0 2 0	0 2 1	0 2 2	0 2 3	0 2 6	0 2 7	0 2 8	0 3 0	Total Tissues/ Tumors
Respiratory System																
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Special Senses System																
None																
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame: 6,250 ppm

Number of Days on Study	0 7 8	1 3 2	1 3 8	1 5 0	1 9 7	2 0 7	2 7 1	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	
Carcass ID Number	0 3 9	0 3 3	0 4 3	0 3 7	0 3 4	0 4 2	0 3 1	0 3 2	0 3 5	0 3 6	0 3 8	0 4 0	0 4 1	0 4 4	0 4 5	Total Tissues/ Tumors
Alimentary System																
Liver	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Salivary glands			+			+										2
Duct, carcinoma			X			X										2
Stomach, forestomach	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Squamous cell papilloma															X	1
Squamous cell papilloma, multiple							X									1
Stomach, glandular	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Tooth							+	+								2
Odontogenic tumor							X	X								2
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Adrenal medulla	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Pituitary gland	A	M	M	I	+	+	I	+	+	+	I	M	I	I	+	6
Thyroid gland	M	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
General Body System																
None																
Genital System																
Coagulating gland															+	1
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Preputial gland										+						1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Lymph node, mandibular	A	+	+	+	+	+	+	+	+	+	+	M	+	+	+	13
Lymph node, mesenteric	A	+	+	M	+	+	+	+	+	+	+	+	+	+	+	13
Lymph node, mediastinal	M	M	+	+	+	+	M	M	+	+	+	+	+	+	+	11
Spleen	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Thymus	M	M	+	+	+	M	+	+	+	+	+	+	+	+	+	12
Integumentary System																
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Musculoskeletal System																
None																
Nervous System																
Brain	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame: 6,250 ppm

Number of Days on Study	0 7 8	1 3 2	1 3 8	1 5 0	1 9 7	2 0 7	2 7 1	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	
Carcass ID Number	0 3 9	0 3 3	0 4 3	0 3 7	0 3 4	0 4 2	0 3 1	0 3 2	0 3 5	0 3 6	0 3 8	0 4 0	0 4 1	0 4 4	0 4 5		Total Tissues/ Tumors
Respiratory System																	
Lung	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Alveolar/bronchiolar adenoma													X		X		2
Special Senses System																	
None																	
Urinary System																	
Kidney	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+		13
Systemic Lesions																	
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Leukemia erythrocytic					X												1
Lymphoma malignant							X										1

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame: 12,500 ppm

Number of Days on Study	1 9 9	2 0 6	2 7 1	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	
Carcass ID Number	0 5 8	0 5 0	0 4 6	0 4 7	0 4 8	0 4 9	0 5 1	0 5 2	0 5 3	0 5 4	0 5 5	0 5 6	0 5 7	0 5 9	0 5 0	0 6 0	Total Tissues/ Tumors
Alimentary System																	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Squamous cell papilloma						X		X			X						3
Squamous cell papilloma, multiple			X	X													2
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Tooth			+							+							2
Odontogenic tumor			X							X							2
Cardiovascular System																	
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																	
Adrenal cortex	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Adrenal medulla	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Pituitary gland	M	+	+	+	I	+	+	I	+	+	I	+	+	+	+	+	11
Thyroid gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
General Body System																	
None																	
Genital System																	
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Testes	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Hematopoietic System																	
Lymph node, mandibular	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mesenteric	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mediastinal	+	+	+	+	+	M	+	M	M	+	I	+	+	+	M		10
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	A	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Integumentary System																	
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Musculoskeletal System																	
None																	
Nervous System																	
Brain	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Respiratory System																	
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Alveolar/bronchiolar adenoma				X													1

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame: 12,500 ppm

Number of Days on Study	1 9 9	2 0 6	2 7 1	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	
Carcass ID Number	0 5 8	0 5 0	0 4 6	0 4 7	0 4 8	0 4 9	0 5 1	0 5 2	0 5 3	0 5 4	0 5 5	0 5 6	0 5 7	0 5 9	0 6 0	Total Tissues/ Tumors
Special Senses System																
None																
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Leukemia erythrocytic	X	X														2

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame: 25,000 ppm

Number of Days on Study	0 9 6	1 3 8	2 3 9	2 6 1	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	
Carcass ID Number	0 6 1	0 7 5	0 6 8	0 7 2	0 6 2	0 6 3	0 6 4	0 6 5	0 6 6	0 6 7	0 6 9	0 7 0	0 7 1	0 7 3	0 7 4		Total Tissues/ Tumors
Alimentary System																	
Liver	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Salivary glands		+	+														2
Duct, carcinoma		X															1
Stomach, forestomach	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Squamous cell papilloma									X			X	X	X			4
Squamous cell papilloma, multiple					X	X											2
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Tooth				+	+												2
Odontogenic tumor				X	X												2
Cardiovascular System																	
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Endocrine System																	
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Pituitary gland	A	+	+	+	+	+	M	+	+	+	+	+	+	+	+		13
Thyroid gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
General Body System																	
None																	
Genital System																	
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Seminal vesicle							+										1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Hematopoietic System																	
Lymph node, mandibular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Lymph node, mediastinal	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Thymus	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Integumentary System																	
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M		
Skin								+									1
Squamous cell papilloma								X									1
Musculoskeletal System																	
None																	
Nervous System																	
Brain	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame: 25,000 ppm

Number of Days on Study	0 9 6	1 3 8	2 3 9	2 6 1	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	
Carcass ID Number	0 6 1	0 7 5	0 6 8	0 7 2	0 6 2	0 6 3	0 6 4	0 6 5	0 6 6	0 6 7	0 6 9	0 7 0	0 7 1	0 7 3	0 7 4	0 7 4	Total Tissues/ Tumors
Respiratory System																	
Lung	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Alveolar/bronchiolar adenoma														X	X		2
Special Senses System																	
Zymbal's gland			+														1
Carcinoma			X														1
Urinary System																	
Kidney	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Systemic Lesions																	
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame: 50,000 ppm

Number of Days on Study	1 4 1	1 8 0	1 8 0	2 0 0	2 7 1	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	
Carcass ID Number	0 8 9	0 8 4	0 9 0	0 8 0	0 8 3	0 7 6	0 7 7	0 7 8	0 7 9	0 8 1	0 8 2	0 8 5	0 8 6	0 8 7	0 8 8	Total Tissues/ Tumors
Alimentary System																
Esophagus	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Gallbladder	A	+	+	+	+	M	+	M	+	+	+	+	+	+	+	12
Intestine large, colon	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Intestine large, rectum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Intestine large, cecum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Intestine small, duodenum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Intestine small, ileum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pancreas	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Salivary glands	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Duct, carcinoma					X											1
Stomach, forestomach	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Squamous cell papilloma		X					X	X	X					X		5
Squamous cell papilloma, multiple													X			1
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Tongue	A	+	+	+	+	+	+	+	+	+	+	+	M	+	+	13
Tooth		+	+	+	+				+				+			6
Odontogenic tumor		X	X	X	X				X				X			6
Cardiovascular System																
Blood vessel	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	A	+	+	+	+	+	+	+	+	+	+	+	+	+	I	13
Adrenal medulla	A	+	+	+	+	+	+	+	+	+	+	+	+	+	M	13
Islets, pancreatic	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Parathyroid gland	A	M	M	M	+	M	+	M	M	+	M	M	M	M	+	4
Pituitary gland	A	+	I	M	+	+	+	I	+	+	+	+	I	+	I	9
Thyroid gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
General Body System																
None																
Genital System																
Coagulating gland												+				1
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Penis				+												1
Preputial gland	A	+	+	+	+	+	+	M	+	+	+	+	M	+	+	12
Prostate	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Seminal vesicle	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame: 50,000 ppm

Number of Days on Study	1 4 1	1 8 0	1 8 0	2 0 0	2 7 1	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	
Carcass ID Number	0 8 9	0 8 4	0 9 0	0 8 0	0 8 3	0 7 6	0 7 7	0 7 8	0 7 9	0 8 1	0 8 2	0 8 5	0 8 6	0 8 7	0 8 8	0 8 8	Total Tissues/ Tumors
Hematopoietic System																	
Bone marrow	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mandibular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mesenteric	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mediastinal	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Thymus	A	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	12
Integumentary System																	
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Musculoskeletal System																	
Bone	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Nervous System																	
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Respiratory System																	
Larynx	M	M	M	M	M	+	M	M	+	I	M	M	M	M	M		2
Lung	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Alveolar/bronchiolar adenoma						X				X							2
Nose	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Trachea	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Special Senses System																	
Eye	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adenoma						X											1
Zymbal's gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Urinary System																	
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Urinary bladder	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Systemic Lesions																	
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymphoma malignant				X													1

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Lung: Alveolar/bronchiolar Adenoma						
Overall rate[a]	0/14 (0%)	0/15 (0%)	2/14 (14%)	1/15 (7%)	2/14 (14%)	2/14 (14%)
Adjusted rate[b]	0.0%	0.0%	19.7%	7.3%	15.8%	16.8%
Terminal rate[c]	0/9 (0%)	0/12 (0%)	2/8 (25%)	1/12 (8%)	2/11 (18%)	2/10 (20%)
First incidence (days)	—[e]	—[f]	274 (T)	274 (T)	274 (T)	274 (T)
Poly-3 test[d]	P=0.098	—	P=0.187	P=0.523	P=0.236	P=0.222
Salivary Glands: Carcinoma						
Overall rate	0/15 (0%)	0/15 (0%)	2/15 (13%)	0/15 (0%)	1/15 (7%)	1/15 (7%)
Adjusted rate	0.0%	0.0%	17.2%	0.0%	7.4%	8.3%
Terminal rate	0/9 (0%)	0/12 (0%)	0/8 (0%)	0/12 (0%)	0/11 (0%)	0/10 (0%)
First incidence (days)	—	—	138	—	138	271
Poly-3 test	P=0.326	—	P=0.211	—	P=0.515	P=0.492
Skin: Squamous Cell Papilloma						
Overall rate	0/15 (0%)	2/15 (13%)	0/15 (0%)	0/15 (0%)	1/15 (7%)	0/15 (0%)
Adjusted rate	0.0%	14.0%	0.0%	0.0%	7.9%	0.0%
Terminal rate	0/9 (0%)	2/12 (17%)	0/8 (0%)	0/12 (0%)	1/11 (9%)	0/10 (0%)
First incidence (days)	—	274 (T)	—	—	274 (T)	—
Poly-3 test	P=0.404N	P=0.261	—	—	P=0.502	—
Stomach (Forestomach): Squamous Cell Papilloma						
Overall rate	8/15 (53%)	5/15 (33%)	2/15 (13%)	5/15 (33%)	6/15 (40%)	6/15 (40%)
Adjusted rate	63.0%	34.9%	19.6%	36.2%	47.3%	47.0%
Terminal rate	5/9 (56%)	5/12 (42%)	1/8 (13%)	4/12 (33%)	6/11 (55%)	5/10 (50%)
First incidence (days)	271	274 (T)	271	271	274 (T)	180
Poly-3 test	P=0.456	P=0.139N	P=0.039N	P=0.157N	P=0.344N	P=0.338N
Tooth: Odontogenic Tumor						
Overall rate	3/15 (20%)	4/15 (27%)	2/15 (13%)	2/15 (13%)	2/15 (13%)	6/15 (40%)
Adjusted rate	22.0%	26.7%	19.6%	14.5%	15.6%	42.4%
Terminal rate	0/9 (0%)	1/12 (8%)	1/8 (13%)	1/12 (8%)	1/11 (9%)	2/10 (20%)
First incidence (days)	76	198	271	271	261	180
Poly-3 test	P=0.128	P=0.556	P=0.635N	P=0.494N	P=0.529N	P=0.230
All Organs: Erythrocytic Leukemia						
Overall rate	1/15 (7%)	0/15 (0%)	1/15 (7%)	2/15 (13%)	0/15 (0%)	0/15 (0%)
Adjusted rate	7.6%	0.0%	9.2%	13.4%	0.0%	0.0%
Terminal rate	0/9 (0%)	0/12 (0%)	0/8 (0%)	0/12 (0%)	0/11 (0%)	0/10 (0%)
First incidence (days)	197	—	197	199	—	—
Poly-3 test	P=0.296N	P=0.484N	P=0.716	P=0.544	P=0.508N	P=0.518N
All Organs: Benign Neoplasms						
Overall rate	8/15 (53%)	6/15 (40%)	3/15 (20%)	5/15 (33%)	8/15 (53%)	8/15 (53%)
Adjusted rate	63.0%	41.9%	29.3%	36.2%	63.0%	62.6%
Terminal rate	5/9 (56%)	6/12 (50%)	2/8 (25%)	4/12 (33%)	8/11 (73%)	7/10 (70%)
First incidence (days)	271	274 (T)	271	271	274 (T)	180
Poly-3 test	P=0.167	P=0.239N	P=0.111N	P=0.157N	P=0.659N	P=0.651N
All Organs: Malignant Neoplasms						
Overall rate	1/15 (7%)	0/15 (0%)	4/15 (27%)	2/15 (13%)	2/15 (13%)	2/15 (13%)
Adjusted rate	7.6%	0.0%	32.5%	13.4%	14.4%	15.7%
Terminal rate	0/9 (0%)	0/12 (0%)	0/8 (0%)	0/12 (0%)	0/11 (0%)	0/10 (0%)
First incidence (days)	197	—	138	199	138	200
Poly-3 test	P=0.371	P=0.484N	P=0.136	P=0.544	P=0.518	P=0.486

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
All Organs: Benign or Malignant Neoplasms						
Overall rate	10/15 (67%)	9/15 (60%)	7/15 (47%)	8/15 (53%)	11/15 (73%)	11/15 (73%)
Adjusted rate	69.9%	60.0%	56.9%	53.3%	78.3%	77.8%
Terminal rate	5/9 (56%)	6/12 (50%)	3/8 (38%)	5/12 (42%)	8/11 (73%)	7/10 (70%)
First incidence (days)	76	198	138	199	138	180
Poly-3 test	P=0.146	P=0.432N	P=0.387N	P=0.296N	P=0.468	P=0.480

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

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame[a]

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Disposition Summary						
Animals initially in study	15	15	15	15	15	15
Early deaths						
Moribund	3	3	4	2	3	4
Natural deaths	3		3	1	1	1
Survivors						
Terminal sacrifice	9	12	8	12	11	10
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Liver	(15)	(15)	(14)	(15)	(14)	(15)
Hematopoietic cell proliferation				1 (7%)		1 (7%)
Infiltration cellular, focal, lymphocyte	1 (7%)					1 (7%)
Inflammation, acute, focal	1 (7%)					
Necrosis, focal						2 (13%)
Stomach, forestomach	(12)	(15)	(13)	(14)	(14)	(14)
Epithelium, hyperplasia	1 (8%)					
Stomach, glandular	(12)	(15)	(13)	(14)	(14)	(14)
Inflammation, acute						1 (7%)
Cardiovascular System						
None						
Endocrine System						
Adrenal cortex	(13)	(15)	(13)	(14)	(15)	(13)
Atrophy	10 (77%)	12 (80%)	8 (62%)	9 (64%)	8 (53%)	6 (46%)
Hypertrophy, focal	9 (69%)	5 (33%)	5 (38%)	6 (43%)	7 (47%)	5 (38%)
Subcapsular, hyperplasia, focal						1 (8%)
Thyroid gland	(13)	(15)	(13)	(14)	(14)	(14)
Follicle, cyst	2 (15)%)					
General Body System						
None						

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

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Genital System						
Coagulating gland			(1)			(1)
Cyst			1 (100%)			
Epididymis	(15)	(15)	(15)	(15)	(15)	(15)
Bilateral, aspermia		1 (7%)				
Bilateral, spermatozoa, degeneration				1 (7%)		1 (7%)
Unilateral, aspermia	1 (7%)	3 (20%)	4 (27%)	2 (13%)	3 (20%)	2 (13%)
Unilateral, cyst	1 (7%)		1 (7%)			1 (7%)
Unilateral, inflammation, chronic active			2 (13%)			
Unilateral, duct, hyperplasia, regenerative	1 (7%)				1 (7%)	
Unilateral, spermatozoa, degeneration						1 (7%)
Preputial gland	(12)	(1)	(1)			(12)
Cyst	1 (8%)	1 (100%)	1 (100%)			
Prostate	(13)	(15)	(15)	(15)	(15)	(14)
Inflammation, acute, focal		1 (7%)				
Seminal vesicle	(13)				(1)	(14)
Dilatation					1 (100%)	
Testes	(15)	(15)	(15)	(14)	(15)	(15)
Cyst			1 (7%)	1 (7%)	1 (7%)	
Bilateral, germinal epithelium, degeneration		2 (13%)		1 (7%)		1 (7%)
Unilateral, cyst	1 (7%)	2 (13%)	3 (20%)	2 (14)%)	2 (13%)	1 (7%)
Unilateral, germinal epithelium, degeneration	1 (7%)	2 (13%)	4 (27%)	2 (14)%)	3 (20%)	3 (20%)
Hematopoietic System						
Bone marrow	(14)					(14)
Hyperplasia						2 (14%)
Lymph node, mandibular	(12)	(15)	(13)	(14)	(14)	(14)
Hyperplasia		2 (13%)		2 (14)%)		
Hyperplasia, plasma cell	1 (8%)					1 (7%)
Necrosis	2 (17%)					
Lymph node, mesenteric	(13)	(15)	(13)	(14)	(15)	(14)
Hyperplasia			1 (8%)			
Necrosis	1 (8%)					
Lymph node, mediastinal	(11)	(13)	(11)	(10)	(14)	(13)
Hyperplasia				1 (10%)		
Spleen	(14)	(15)	(13)	(15)	(14)	(14)
Hematopoietic cell proliferation	3 (21%)	3 (20%)	8 (62%)	2 (13%)	8 (57%)	4 (29%)
Pigmentation	9 (64%)	12 (80%)	8 (62%)	12 (80%)	13 (93%)	11 (79%)
Lymphoid follicle, depletion cellular		1 (7%)				
Thymus	(12)	(14)	(12)	(13)	(14)	(12)
Atrophy, diffuse	1 (8%)		1 (8%)		4 (29%)	
Atrophy, focal				1 (8%)	2 (14)%)	2 (17%)
Integumentary System						
Skin	(15)	(2)			(1)	(15)
Control epidermis, hyperplasia, focal					1 (100%)	
Musculoskeletal System						
None						

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Nervous System						
Brain	(15)	(15)	(13)	(14)	(14)	(15)
Cortex, cerebrum, degeneration, focal						1 (7%)
Respiratory System						
Lung	(14)	(15)	(14)	(15)	(14)	(14)
Hemorrhage, focal		3 (20%)			1 (7%)	
Alveolar epithelium, hyperplasia, focal						1 (7%)
Special Senses System						
Eye	(12)					(14)
Retina, atrophy	12 (100%)					14 (100%)
Urinary System						
Kidney	(15)	(15)	(13)	(15)	(14)	(15)
Dilatation, diffuse			1 (8%)			
Nephropathy	3 (20%)					1 (7%)
Renal tubule, cyst						1 (7%)
Renal tubule, dilatation, diffuse				1 (7%)		1 (7%)
Renal tubule, dilatation, focal	1 (7%)		1 (8%)			

APPENDIX B
SUMMARY OF LESIONS
IN FEMALE Tg.AC HEMIZYGOUS MICE
IN THE 9-MONTH FEED STUDY
OF ASPARTAME

TABLE B1
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame[a]

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Disposition Summary						
Animals initially in study	15	15	15	15	15	15
Early deaths						
Moribund	3	2	3	4	3	2
Natural deaths	1	3	3	2	1	5
Survivors						
Terminal sacrifice	11	10	9	9	11	8
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Liver	(15)	(15)	(14)	(15)	(14)	(14)
Sarcoma stromal, metastatic, uterus				1 (7%)		
Pancreas	(15)					(13)
Salivary glands	(15)			(1)		(13)
Duct, carcinoma				1 (100%)		1 (8%)
Stomach, forestomach	(15)	(13)	(13)	(15)	(14)	(11)
Squamous cell papilloma	2 (13%)	6 (46%)	1 (8%)	7 (47%)	3 (21%)	5 (45%)
Squamous cell papilloma, multiple	2 (13%)	3 (23%)	3 (23%)		4 (29%)	
Tooth	(4)	(4)	(2)	(5)	(5)	(1)
Odontogenic tumor	4 (100%)					1 (100%)
Odontoma		4 (100%)	2 (100%)	5 (100%)	5 (100%)	
Cardiovascular System						
Heart	(15)	(15)	(15)	(15)	(15)	(15)
Endocrine System						
Adrenal cortex	(15)	(12)	(13)	(13)	(14)	(15)
Adrenal medulla	(15)	(12)	(13)	(13)	(14)	(14)
Islets, pancreatic	(15)					(11)
Pituitary gland	(13)	(11)	(12)	(10)	(11)	(9)
Thyroid gland	(15)	(13)	(12)	(15)	(14)	(11)
General Body System						
None						
Genital System						
Ovary	(15)	(15)	(13)	(14)	(14)	(14)
Uterus	(15)	(13)	(13)	(12)	(14)	(14)
Sarcoma stromal				1 (8%)		
Hematopoietic System						
Bone marrow	(15					(12)
Lymph node	(1)			(1)		
Pancreatic, sarcoma stromal, metastatic, uterus				1 (100%)		
Lymph node, mediastinal	(12)	(11)	(11)	(12)	(11)	(11)
Carcinoma, metastatic, salivary glands				1 (8%)		
Spleen	(15	(14)	(14)	(12	(14	(14)

TABLE B1
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Integumentary System						
Mammary gland	(15)	(13)	(13)	(14)	(14)	(11)
Skin	(15)	(3)	(1)		(3)	(15)
Squamous cell papilloma		2 (67%)	1 (100%)			
Vulva, squamous cell papilloma	1 (7%)	1 (33%)			2 (67%)	
Musculoskeletal System						
None						
Nervous System						
Brain	(15)	(12)	(13)	(13)	(14)	(11)
Respiratory System						
Lung	(15)	(14)	(14)	(15)	(14)	(14)
Alveolar/bronchiolar adenoma	1 (7%)					
Carcinoma, metastatic, salivary glands				1 (7%)		
Nose	(15)					(13)
Special Senses System						
None						
Urinary System						
Kidney	(15)	(12)	(13)	(14)	(14)	(14)
Systemic Lesions						
Multiple organs[b]	(15)	(15)	(15)	(15)	(15)	(15)
Leukemia erythrocytic	1 (7%)	3 (20%)	1 (7%)			1 (7%)
Neoplasm Summary						
Total animals with primary neoplasms[c]	8	12	8	11	11	7
Total primary neoplasms	11	19	8	14	14	8
Total animals with benign neoplasms	4	12	7	9	11	5
Total benign neoplasms	6	16	7	12	14	5
Total animals with malignant neoplasms	1	3	1	2		2
Total malignant neoplasms	1	3	1	2		2
Total animals with metastatic neoplasms				2		
Total metastatic neoplasms				4		
Total animals with uncertain neoplasms-benign or malignant	4					1
Total uncertain neoplasms	4					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
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame: 0 ppm

Number of Days on Study	1 0 9	2 0 0	2 4 2	2 7 0	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	
Carcass ID Number	1 1 7	1 1 1	1 0 6	1 1 4	1 0 7	1 0 8	1 0 9	1 1 0	1 1 2	1 1 3	1 1 5	1 1 6	1 1 8	1 1 9	1 2 0	Total Tissues/ Tumors
Alimentary System																
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Gallbladder	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Squamous cell papilloma					X	X										2
Squamous cell papilloma, multiple								X							X	2
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Tooth	+	+	+	+												4
Odontogenic tumor	X	X	X	X												4
Cardiovascular System																
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Parathyroid gland	M	M	M	M	+	M	+	M	+	+	M	M	M	M	M	4
Pituitary gland	+	I	+	+	+	+	M	+	+	+	+	+	+	+	+	13
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
Tissue nos									+		+					2
Genital System																
Clitoral gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

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

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame: 0 ppm

Number of Days on Study	1 0 9	2 0 0	2 4 2	2 7 0	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	
Carcass ID Number	1 1 7	1 1 1	1 0 6	1 1 4	1 0 7	1 0 8	1 0 9	1 1 0	1 1 2	1 1 3	1 1 5	1 1 6	1 1 8	1 1 9	1 2 0		Total Tissues/ Tumors
Hematopoietic System																	
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Lymph node													+				1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Lymph node, mediastinal	+	M	+	+	+	+	+	I	+	+	+	M	+	+	+		12
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Integumentary System																	
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Vulva, squamous cell papilloma					X												1
Musculoskeletal System																	
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Nervous System																	
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Respiratory System																	
Larynx	M	M	M	M	M	M	M	M	I	M	M	M	M	M	M		
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Alveolar/bronchiolar adenoma					X												1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Special Senses System																	
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Urinary System																	
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Urinary bladder	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+		14
Systemic Lesions																	
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Leukemia erythrocytic	X																1

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame: 3,125 ppm

Number of Days on Study	0 8 1	1 9 2	1 9 8	2 0 6	2 7 1	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	
Carcass ID Number	1 3 2	1 2 1	1 3 4	1 3 3	1 3 0	1 2 2	1 2 3	1 2 4	1 2 5	1 2 6	1 2 7	1 2 8	1 2 9	1 3 1	1 3 5	Total Tissues/ Tumors
Alimentary System																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	13
Squamous cell papilloma					X	X			X			X	X	X		6
Squamous cell papilloma, multiple		X								X					X	3
Stomach, glandular	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	12
Tooth				+	+	+								+		4
Odontoma				X	X	X								X		4
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	12
Adrenal medulla	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	12
Pituitary gland	+	+	I	A	I	M	+	+	+	+	+	+	+	+	+	11
Thyroid gland	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	13
General Body System																
None																
Genital System																
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Uterus	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	13
Hematopoietic System																
Lymph node, mandibular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mesenteric	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	13
Lymph node, mediastinal	A	M	+	A	+	+	+	+	+	+	+	M	+	+	+	11
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Thymus	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	13
Integumentary System																
Mammary gland	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	13
Skin			+				+						+			3
Squamous cell papilloma			X										X			2
Vulva, squamous cell papilloma							X									1
Musculoskeletal System																
None																
Nervous System																
Brain	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	12
Respiratory System																
Lung	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame: 3,125 ppm

Number of Days on Study	0 8 1	1 9 2	1 9 8	2 0 6	2 7 1	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	
Carcass ID Number	1 3 2	1 2 1	1 3 4	1 3 3	1 3 0	1 2 2	1 2 3	1 2 4	1 2 5	1 2 6	1 2 7	1 2 8	1 2 9	1 3 1	1 3 5	Total Tissues/ Tumors
Special Senses System																
None																
Urinary System																
Kidney	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	12
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Leukemia erythrocytic		X	X	X												3

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame: 6,250 ppm

Number of Days on Study	0 6 1	0 6 7	1 1 3	1 4 8	2 0 0	2 0 7	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	
Carcass ID Number	1 3 8	1 4 8	1 4 1	1 3 9	1 3 7	1 4 9	1 3 6	1 4 0	1 4 2	1 4 3	1 4 4	1 4 5	1 4 6	1 4 7	1 5 0	Total Tissues/ Tumors
Alimentary System																
Liver	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	14
Stomach, forestomach	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	13
Squamous cell papilloma								X								1
Squamous cell papilloma, multiple							X					X	X			3
Stomach, glandular	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	13
Tooth					+	+										2
Odontoma					X	X										2
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	13
Adrenal medulla	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	13
Pituitary gland	M	+	A	A	+	+	+	+	+	+	+	+	+	+	+	12
Thyroid gland	+	+	A	A	+	+	+	+	+	+	M	+	+	+	+	12
General Body System																
None																
Genital System																
Ovary	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	13
Uterus	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	13
Hematopoietic System																
Lymph node, mandibular	+	+	A	+	M	M	+	+	+	+	+	+	+	+	+	12
Lymph node, mesenteric	M	+	A	A	+	+	+	+	+	+	+	+	+	+	+	12
Lymph node, mediastinal	+	+	A	A	+	+	+	+	M	+	+	M	+	+	+	11
Spleen	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	14
Thymus	+	+	A	A	M	+	+	+	+	+	+	+	+	+	+	12
Integumentary System																
Mammary gland	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	13
Skin														+		1
Squamous cell papilloma														X		1
Musculoskeletal System																
None																
Nervous System																
Brain	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	13
Respiratory System																
Lung	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	14

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame: 6,250 ppm

Number of Days on Study	0 6 1	0 6 7	1 1 3	1 4 8	2 0 0	2 0 7	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	
Carcass ID Number	1 3 8	1 4 8	1 4 1	1 3 9	1 3 7	1 4 9	1 3 6	1 4 0	1 4 2	1 4 3	1 4 4	1 4 5	1 4 6	1 4 7	1 4 5	Total Tissues/ Tumors
Special Senses System																
None																
Urinary System																
Kidney	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	13
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Leukemia erythrocytic				X												1

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame: 12,500 ppm

Number of Days on Study	0 9 7	1 3 4	1 6 5	1 6 5	1 8 4	2 7 1	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	
Carcass ID Number	1 5 6	1 5 3	1 5 4	1 6 0	1 6 4	1 5 9	1 5 1	1 5 2	1 5 5	1 5 7	1 5 8	1 6 1	1 6 2	1 6 3	1 6 5	Total Tissues/ Tumors
Alimentary System																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Sarcoma stromal, metastatic, uterus											X					1
Salivary glands	+															1
Duct, carcinoma	X															1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Squamous cell papilloma					X	X		X		X		X	X		X	7
Stomach, glandular	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	14
Tooth			+	+	+	+						+				5
Odontoma			X	X	X	X						X				5
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	13
Adrenal medulla	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	13
Pituitary gland	I	M	+	M	+	+	+	+	+	+	+	+	M	+	I	10
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
None																
Genital System																
Ovary	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	14
Uterus	A	+	A	+	+	A	+	+	+	+	+	+	+	+	+	12
Sarcoma stromal											X					1
Hematopoietic System																
Lymph node											+					1
Pancreatic, sarcoma stromal, metastatic, uterus											X					1
Lymph node, mandibular	A	+	A	M	+	+	+	+	+	+	+	+	+	+	+	12
Lymph node, mesenteric	A	A	+	+	+	+	+	+	+	+	I	+	+	+	+	12
Lymph node, mediastinal	+	+	A	+	+	+	+	+	+	+	+	M	+	+	M	12
Carcinoma, metastatic, salivary glands	X															1
Spleen	A	A	+	+	+	A	+	+	+	+	+	+	+	+	+	12
Thymus	M	A	M	+	+	+	+	+	+	+	+	+	+	+	+	12
Integumentary System																
Mammary gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Musculoskeletal System																
None																

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame: 12,500 ppm

Number of Days on Study	0 9 7	1 3 4	1 6 5	1 6 5	1 8 4	2 7 1	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	
Carcass ID Number	1 5 6	1 5 3	1 5 4	1 6 0	1 6 4	1 5 9	1 5 1	1 5 2	1 5 5	1 5 7	1 5 8	1 6 1	1 6 2	1 6 3	1 6 5	Total Tissues/ Tumors
Nervous System																
Brain	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Respiratory System																
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Carcinoma, metastatic, salivary glands	X															1
Special Senses System																
None																
Urinary System																
Kidney	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame: 25,000 ppm

Number of Days on Study	0 2 3	2 6 7	2 6 9	2 7 0	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	
Carcass ID Number	1 7 8	1 6 7	1 7 3	1 6 8	1 6 6	1 6 9	1 7 0	1 7 1	1 7 2	1 7 4	1 7 5	1 7 6	1 7 7	1 7 9	1 8 0	Total Tissues/ Tumors
Alimentary System																
Liver	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Stomach, forestomach	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Squamous cell papilloma				X				X	X							3
Squamous cell papilloma, multiple		X				X	X								X	4
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Tooth		+	+	+								+		+		5
Odontoma		X	X	X								X		X		5
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Adrenal medulla	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Pituitary gland	A	+	+	I	+	+	I	+	I	+	+	+	+	+	+	11
Thyroid gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
General Body System																
None																
Genital System																
Ovary	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Uterus	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Hematopoietic System																
Lymph node, mandibular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mesenteric	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mediastinal	A	+	+	+	M	+	+	+	M	I	+	+	+	+	+	11
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Thymus	A	M	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Integumentary System																
Mammary gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Skin								+			+	+				3
Vulva, squamous cell papilloma								X			X					2
Musculoskeletal System																
None																
Nervous System																
Brain	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Respiratory System																
Lung	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame: 25,000 ppm

Number of Days on Study	0 2 3	2 6 7	2 6 9	2 7 0	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	
Carcass ID Number	1 7 8	1 6 7	1 7 3	1 6 8	1 6 6	1 6 9	1 7 0	1 7 1	1 7 2	1 7 4	1 7 5	1 7 6	1 7 7	1 7 9	1 8 0	Total Tissues/ Tumors
Special Senses System																
None																
Urinary System																
Kidney	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame: 50,000 ppm

Number of Days on Study	0 7 9	0 9 2	0 9 2	1 5 1	1 8 4	2 1 5	2 6 9	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	
Carcass ID Number	1 8 8	1 8 2	1 9 2	1 9 1	1 9 0	1 9 4	1 8 7	1 8 1	1 8 3	1 8 4	1 8 5	1 8 6	1 8 9	1 9 3	1 9 5	Total Tissues/ Tumors
Alimentary System																
Esophagus	+	A	A	+	M	A	+	+	+	+	+	+	+	+	+	11
Gallbladder	A	A	M	A	M	A	+	M	+	M	M	+	+	+	+	6
Intestine large, colon	A	A	A	A	+	A	+	+	+	+	+	+	+	+	+	10
Intestine large, rectum	A	A	A	A	+	A	+	+	+	+	+	+	+	+	+	10
Intestine large, cecum	A	A	A	A	+	A	+	+	+	+	+	+	+	+	+	10
Intestine small, duodenum	A	A	A	A	+	A	+	+	+	+	+	+	+	+	+	10
Intestine small, jejunum	A	A	A	A	+	A	+	+	+	+	+	+	+	+	+	10
Intestine small, ileum	A	A	A	A	+	A	+	+	+	+	+	+	+	+	+	10
Liver	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	14
Pancreas	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	13
Salivary glands	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	13
Duct, carcinoma							X									1
Stomach, forestomach	+	A	A	A	+	A	+	+	+	+	+	+	+	+	+	11
Squamous cell papilloma					X			X		X	X				X	5
Stomach, glandular	A	A	A	A	+	A	+	+	+	+	+	+	+	+	+	10
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Tooth					+											1
Odontogenic tumor					X											1
Cardiovascular System																
Blood vessel	A	A	A	+	+	A	+	+	M	+	+	+	+	+	+	10
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	14
Islets, pancreatic	A	A	A	A	+	+	+	+	+	+	+	+	+	+	+	11
Parathyroid gland	M	M	M	A	M	M	I	M	M	M	M	+	M	+	+	3
Pituitary gland	M	M	M	A	+	A	+	+	+	I	+	+	+	+	+	9
Thyroid gland	+	A	A	A	+	+	+	+	+	+	M	+	+	+	+	11
General Body System																
None																
Genital System																
Clitoral gland	+	+	A	M	M	A	+	+	M	+	+	+	+	+	+	10
Ovary	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	14
Uterus	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	14
Vagina	A	A	A	+	+	A	+	+	+	+	+	+	+	+	+	11

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame: 50,000 ppm

Number of Days on Study	0 7 9	0 9 2	0 9 2	1 5 1	1 8 4	2 1 5	2 6 9	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	
Carcass ID Number	1 8 8	1 8 2	1 9 2	1 9 1	1 9 0	1 9 4	1 8 7	1 8 1	1 8 3	1 8 4	1 8 5	1 8 6	1 8 9	1 8 3	1 9 5	Total Tissues/ Tumors
Hematopoietic System																
Bone marrow	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	12
Lymph node, mandibular	A	+	A	+	+	A	+	+	+	+	+	+	+	+	+	12
Lymph node, mesenteric	A	A	A	A	+	A	+	+	+	+	+	+	+	M	+	9
Lymph node, mediastinal	A	A	A	M	+	+	+	+	+	+	+	+	+	+	+	11
Spleen	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	14
Thymus	+	A	A	A	+	A	+	+	+	+	+	+	+	+	+	11
Integumentary System																
Mammary gland	A	A	A	+	+	+	+	+	M	+	+	+	+	+	+	11
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Musculoskeletal System																
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Skeletal muscle	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	14
Nervous System																
Brain	A	A	A	+	+	A	+	+	+	+	+	+	+	+	+	11
Respiratory System																
Larynx	M	M	M	M	M	M	M	M	I	M	M	M	I	M	I	
Lung	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	14
Nose	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	13
Trachea	A	A	A	A	+	A	+	+	+	+	+	+	+	+	+	10
Special Senses System																
Eye	A	A	A	A	+	A	+	+	+	+	+	+	+	+	+	10
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Zymbal's gland	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	13
Urinary System																
Kidney	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Urinary bladder	A	A	A	A	+	A	+	+	+	+	+	+	+	+	+	10
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Leukemia erythrocytic						X										1

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Lung: Alveolar/bronchiolar Adenoma						
Overall rate[a]	1/15 (7%)	0/14 (0%)	0/14 (0%)	0/15 (0%)	0/14 (0%)	0/14 (0%)
Adjusted rate[b]	7.7%	0.0%	0.0%	0.0%	0.0%	0.0%
Terminal rate[c]	1/11 (9%)	0/10 (0%)	0/9 (0%)	0/9 (0%)	0/11 (0%)	0/8 (0%)
First incidence (days)	275 (T)	—[e]	—	—	—	—
Poly-3 test[d]	P=0.332N	P=0.516N	P=0.553N	P=0.537N	P=0.489N	P=0.554N
Salivary Glands: Carcinoma						
Overall rate	0/15 (0%)	0/15 (0%)	0/15 (0%)	1/15 (7%)	0/15 (0%)	1/15 (7%)
Adjusted rate	0.0%	0.0%	0.0%	8.5%	0.0%	10.0%
Terminal rate	0/11 (0%)	0/10 (0%)	0/9 (0%)	0/9 (0%)	0/11 (0%)	0/8 (0%)
First incidence (days)	—	—	—	97	—	269
Poly-3 test	P=0.204	—[f]	—	P=0.480	—	P=0.448
Skin: Squamous Cell Papilloma						
Overall rate	1/15 (7%)	3/15 (20%)	1/15 (7%)	0/15 (0%)	2/15 (13%)	0/15 (0%)
Adjusted rate	7.7%	23.5%	9.9%	0.0%	14.5%	0.0%
Terminal rate	1/11 (9%)	2/10 (20%)	1/9 (11%)	0/9 (0%)	2/11 (18%)	0/8 (0%)
First incidence (days)	275 (T)	198	275 (T)	—	275 (T)	—
Poly-3 test	P=0.244N	P=0.286	P=0.700	P=0.537N	P=0.519	P=0.553N
Stomach (Forestomach): Squamous Cell Papilloma						
Overall rate	4/15 (27%)	9/15 (60%)	4/15 (27%)	7/15 (47%)	7/15 (47%)	5/15 (33%)
Adjusted rate	30.6%	70.2%	39.8%	60.4%	50.2%	46.8%
Terminal rate	4/11 (36%)	7/10 (70%)	4/9 (44%)	5/9 (56%)	5/11 (46%)	4/8 (50%)
First incidence (days)	275 (T)	192	275 (T)	184	267	184
Poly-3 test	P=0.539	P=0.042	P=0.493	P=0.136	P=0.261	P=0.352
Tooth: Odontogenic Tumor						
Overall rate	4/15 (27%)	4/15 (27%)	2/15 (13%)	5/15 (33%)	5/15 (33%)	1/15 (7%)
Adjusted rate	26.7%	31.4%	17.8%	38.0%	35.7%	9.4%
Terminal rate	0/11 (0%)	2/10 (20%)	0/9 (0%)	1/9 (11%)	2/11 (18%)	0/8 (0%)
First incidence (days)	109	206	200	165	267	184
Poly-3 test	P=0.296N	P=0.556	P=0.475N	P=0.409	P=0.452	P=0.287N
All Organs: Erythrocytic Leukemia						
Overall rate	1/15 (7%)	3/15 (20%)	1/15 (7%)	0/15 (0%)	0/15 (0%)	1/15 (7%)
Adjusted rate	7.1%	21.5%	9.2%	0.0%	0.0%	9.5%
Terminal rate	0/11 (0%)	0/10 (0%)	0/9 (0%)	0/9 (0%)	0/11 (0%)	0/8 (0%)
First incidence (days)	109	192	148	—	—	215
Poly-3 test	P=0.293N	P=0.296	P=0.703	P=0.550N	P=0.503N	P=0.693
All Organs: Benign Neoplasms						
Overall rate	4/15 (27%)	12/15 (80%)	7/15 (47%)	9/15 (60%)	11/15 (73%)	5/15 (33%)
Adjusted rate	30.6%	85.6%	62.2%	68.4%	78.6%	46.8%
Terminal rate	4/11 (36%)	8/10 (80%)	5/9 (56%)	5/9 (56%)	8/11 (73%)	4/8 (50%)
First incidence (days)	275 (T)	192	200	165	267	184
Poly-3 test	P=0.535N	P=0.002	P=0.120	P=0.053	P=0.010	P=0.352
All Organs: Malignant Neoplasms						
Overall rate	1/15 (7%)	3/15 (20%)	1/15 (7%)	2/15 (13%)	0/15 (0%)	2/15 (13%)
Adjusted rate	7.1%	21.5%	9.2%	16.9%	0.0%	18.9%
Terminal rate	0/11 (0%)	0/10 (0%)	0/9 (0%)	1/9 (11%)	0/11 (0%)	0/8 (0%)
First incidence (days)	109	192	148	97	—	215
Poly-3 test	P=0.564N	P=0.296	P=0.703	P=0.439	P=0.503N	P=0.400

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
All Organs: Benign or Malignant Neoplasms						
Overall rate	8/15 (53%)	12/15 (80%)	8/15 (53%)	11/15 (73%)	11/15 (73%)	7/15 (47%)
Adjusted rate	53.3%	85.6%	66.1%	77.9%	78.6%	62.1%
Terminal rate	4/11 (36%)	8/10 (80%)	5/9 (56%)	6/9 (67%)	8/11 (73%)	4/8 (50%)
First incidence (days)	109	192	148	97	267	184
Poly-3 test	P=0.541	P=0.065	P=0.393	P=0.157	P=0.150	P=0.480

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

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame[a]

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Disposition Summary						
Animals initially in study	15	15	15	15	15	15
Early deaths						
Moribund	3	2	3	4	3	2
Natural deaths	1	3	3	2	1	5
Survivors						
Terminal sacrifice	11	10	9	9	11	8
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Gallbladder	(14)					(6)
Inflammation, chronic active	1 (7%)					
Liver	(15)	(15)	(14)	(15)	(14)	(14)
Infiltration cellular, focal, lymphocyte		1 (7%)				
Infiltration cellular, focal, polymorphonuclear				1 (7%)		
Inflammation, acute, focal	1 (7%)				1 (7%)	2 (14%)
Inflammation, chronic active, focal	2 (13%)				1 (7%)	1 (7%)
Necrosis			1 (7%)			
Necrosis, focal	1 (7%)					4 (29%)
Hepatocyte, necrosis, focal		1 (7%)		4 (27%)	1 (7%)	
Stomach, forestomach	(15)	(13)	(13)	(15)	(14)	(11)
Infiltration cellular, focal, lymphocyte			1 (8%)			
Muscularis, inflammation, acute, focal			1 (8%)			
Tongue	(15)					(15)
Ulcer	1 (7%)					
Cardiovascular System						
None						
Endocrine System						
Adrenal cortex	(15)	(12)	(13)	(13)	(14)	(15)
Accessory adrenal cortical nodule	1 (7%)					
Atrophy	3 (20%)	2 (17%)	1 (8%)	4 (31%)	5 (36%)	5 (33%)
Capsule, inflammation, acute, focal					1 (7%)	
Subcapsular, hyperplasia, focal	12 (80%)	6 (50%)	6 (46%)	5 (38%)	6 (43%)	6 (40%)
Zona reticularis, vacuolization cytoplasmic	12 (80%)	9 (75%)	6 (46%)	8 (62%)	9 (64%)	8 (53%)
Pituitary gland	(13)	(11)	(12)	(10)	(11)	(9)
Cyst	2 (15%)					
Pars intermedia, hypertrophy					1 (9%)	
Thyroid gland	(15)	(13)	(12)	(15)	(14)	(11)
Follicle, cyst	1 (7%)					
General Body System						
Tissue NOS	(2)					
Pigmentation	1 (50%)					
Abdominal, fat, necrosis, focal	1 (50%)					

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

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Genital System						
Ovary	(15)	(15)	(13)	(14)	(14)	(14)
Atrophy	2 (13%)		1 (8%)			
Cyst, focal					1 (7%)	
Hemorrhage, focal					1 (7%)	
Uterus	(15)	(13)	(13)	(12)	(14)	(14)
Cyst, focal				1 (8%)		
Hydrometra					1 (7%)	
Endometrium, hyperplasia, cystic	10 (67%)	8 (62%)	9 (69%)	10 (83%)	10 (71%)	10 (71%)
Hematopoietic System						
Lymph node	(1)			(1)		
Renal, hyperplasia	1 (100%)					
Lymph node, mandibular	(15)	(14)	(12)	(12)	(14)	(12)
Hyperplasia		2 (14%)		2 (17%)	2 (14%)	
Hyperplasia, plasma cell	2 (13%)					1 (8%)
Inflammation, acute, focal					1 (7%)	
Lymph node, mediastinal	(12)	(11)	(11)	(12)	(11)	(11)
Hyperplasia	1 (8%)				1 (9%)	
Spleen	(15)	(14)	(14)	(12)	(14)	(14)
Accessory spleen		1 (7%)				
Hematopoietic cell proliferation	10 (67%)	4 (29%)	7 (50%)	6 (50%)	8 (57%)	5 (36%)
Pigmentation	10 (67%)	10 (71%)	11 (79%)	10 (83%)	11 (79%)	9 (64%)
Thymus	(15)	(13)	(12)	(12)	(13)	(11)
Atrophy, diffuse	3 (20%)	1 (8%)	1 (8%)	2 (17%)	2 (15%)	2 (18%)
Atrophy, focal	2 (13%)	1 (8%)	1 (8%)			1 (9%)
Hyperplasia, diffuse		1 (8%)				
Integumentary System						
Skin	(15)	(3)	(1)		(3)	(15)
Lip, control epidermis, hyperplasia, focal					1 (33%)	
Musculoskeletal System						
None						
Nervous System						
Brain	(15)	(12)	(13)	(13)	(14)	(11)
Abscess, focal					1 (7%)	
Inflammation, acute, focal					1 (7%)	
Cerebellum, corpus callosum, pons, vacuolization cytoplasmic, focal			1 (8%)			
Cortex, cerebrum, degeneration, focal						1 (9%)
Cortex, cerebrum, neuron, necrosis			1 (8%)			
Medulla, vacuolization cytoplasmic					1 (7%)	
Pyramidal cell, hippocampus, necrosis			1 (8%)			

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Respiratory System						
Lung	(15)	(14)	(14)	(15)	(14)	(14)
Hemorrhage, focal	1 (7%)					
Infiltration cellular, focal, lymphocyte	1 (7%)					
Inflammation, chronic active, focal	1 (7%)					
Alveolar epithelium, hyperplasia, focal	1 (7%)	1 (7%)				
Perivascular, infiltration cellular, lymphocyte				1 (7%)	1 (7%)	
Special Senses System						
Eye	(15)					(10)
Retina, atrophy	15 (100%)					10 (100%)
Harderian gland	(15)					(15)
Inflammation, chronic active						1 (7%)
Urinary System						
Kidney	(15)	(12)	(13)	(14)	(14)	(14)
Infiltration cellular, focal, lymphocyte	1 (7%)					
Inflammation, acute, focal					1 (7%)	
Mineralization, focal						1 (7%)
Nephropathy	1 (7%)					1 (7%)
Renal tubule, dilatation, focal	1 (7%)	2 (17%)	1 (8%)			1 (7%)

APPENDIX C
SUMMARY OF LESIONS
IN MALE p53 HAPLOINSUFFICIENT MICE
IN THE 9-MONTH FEED STUDY
OF ASPARTAME

TABLE C1
Summary of the Incidence of Neoplasms in Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame[a]

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Disposition Summary						
Animals initially in study	15	15	15	15	15	15
Early deaths						
Moribund			1			1
Natural deaths	1		1		1	
Survivors						
Terminal sacrifice	14	15	13	15	14	14
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Intestine small, duodenum	(14)					(15)
Polyp adenomatous						1 (7%)
Liver	(15)	(15)	(15)	(15)	(14)	(15)
Salivary glands	(15)	(1)	(1)		(1)	(15)
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
Prostate	(14)	(15)	(15)	(15)	(14)	(15)
Sarcoma					1 (7%)	1 (7%)
Hematopoietic System						
Lymph node, mandibular	(14)	(15)	(14)	(15)	(14)	(15)
Lymph node, mesenteric	(14)	(14)	(15)	(13)	(14)	(15)
Lymph node, mediastinal	(13)	(14)	(14)	(13)	(12)	(13)
Spleen	(15)	(15)	(15)	(15)	(14)	(15)
Thymus	(14)	(15)	(15)	(14)	(15)	(14)
Integumentary System						
Skin	(15)					(15)
Subcutaneous tissue, sarcoma						1 (7%)
Musculoskeletal System						
None						
Nervous System						
None						

TABLE C1
Summary of the Incidence of Neoplasms in Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Respiratory System						
Lung	(15)	(15)	(15)	(15)	(14)	(15)
Special Senses System						
None						
Urinary System						
Kidney	(14)	(15)	(15)	(15)	(14)	(15)
Systemic Lesions						
Multiple organs[b]	(15)	(15)	(15)	(15)	(15)	(15)
Lymphoma malignant			2 (13%)			
Neoplasm Summary						
Total animals with primary neoplasms[c]			2		1	3
Total primary neoplasms			2		1	3
Total animals with benign neoplasms						1
Total benign neoplasms						1
Total animals with malignant neoplasms			2		1	2
Total malignant neoplasms			2		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 C2
Individual Animal Tumor Pathology of Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame: 0 ppm

Number of Days on Study	1 0 2	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 5	2 7 5	
Carcass ID Number	2 2 0	2 1 1	2 1 3	2 1 4	2 1 5	2 1 6	2 1 7	2 1 9	2 2 1	2 2 2	2 2 3	2 2 4	2 2 5	2 1 2	2 1 8	Total Tissues/ Tumors
Alimentary System																
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Gallbladder	A	I	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Intestine large, colon	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Intestine large, rectum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Intestine large, cecum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Intestine small, duodenum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Intestine small, ileum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pancreas	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Cardiovascular System																
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	14
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Islets, pancreatic	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Parathyroid gland	M	+	+	M	M	M	M	M	+	+	M	+	M	M	+	6
Pituitary gland	I	+	+	+	+	+	I	+	+	+	+	+	+	+	+	13
Thyroid gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
General Body System																
None																
Genital System																
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Preputial gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Prostate	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Seminal vesicle	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

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

TABLE C2
Individual Animal Tumor Pathology of Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame: 0 ppm

Number of Days on Study	1 0 2	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 5	2 7 5	
Carcass ID Number	2 2 0	2 1 1	2 1 3	2 1 4	2 1 5	2 1 6	2 1 7	2 1 9	2 2 1	2 2 2	2 2 3	2 2 4	2 2 5	2 1 2	2 1 8	Total Tissues/ Tumors
Hematopoietic System																
Bone marrow	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mandibular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mesenteric	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mediastinal	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	13
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Integumentary System																
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Musculoskeletal System																
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Nervous System																
Brain	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Peripheral nerve											+					1
Spinal cord											+					1
Respiratory System																
Larynx	M	M	M	M	M	M	M	M	+	M	M	+	+	+	M	4
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Nose	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Trachea	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Special Senses System																
Eye	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Zymbal's gland	+	+	+	+	+	+	+	+	M	+	M	M	+	+	+	12
Urinary System																
Kidney	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Urinary bladder	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE C2
Individual Animal Tumor Pathology of Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame: 3,125 ppm

Number of Days on Study	274	274	274	274	274	274	274	274	275	275	275	275	275	275	275	
Carcass ID Number	22727	22728	23030	23302	23303	23306	23309	24000	22706	22709	23301	23304	23305	23307	23308	Total Tissues/ Tumors
Alimentary System																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Salivary glands							+									1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pituitary gland	+	I	+	+	+	+	+	+	+	+	I	+	+	+	+	13
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
None																
Genital System																
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	14
Lymph node, mediastinal	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	14
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Integumentary System																
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Musculoskeletal System																
None																
Nervous System																
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Respiratory System																
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Special Senses System																
None																

TABLE C2
Individual Animal Tumor Pathology of Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame: 3,125 ppm

Number of Days on Study	274	274	274	274	274	274	274	274	275	275	275	275	275	275	275	
Carcass ID Number	227	228	230	232	233	236	239	240	226	229	231	234	235	237	238	Total Tissues/ Tumors
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE C2
Individual Animal Tumor Pathology of Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame: 6,250 ppm

Number of Days on Study	1 1 4	2 1 0	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 5	2 7 5	2 7 5	2 7 5	
Carcass ID Number	2 4 6	2 4 9	2 4 1	2 4 2	2 4 3	2 4 5	2 4 8	2 5 1	2 5 2	2 5 4	2 5 5	2 4 4	2 4 7	2 5 0	2 5 3		Total Tissues/ Tumors
Alimentary System																	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Salivary glands		+															1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Cardiovascular System																	
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Endocrine System																	
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Pituitary gland	+	I	I	I	+	+	+	+	+	+	+	+	I	+	+		11
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
General Body System																	
None																	
Genital System																	
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Hematopoietic System																	
Lymph node, mandibular	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+		14
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Lymph node, mediastinal	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+		14
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Integumentary System																	
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M		
Musculoskeletal System																	
None																	
Nervous System																	
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Respiratory System																	
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Special Senses System																	
Eye						+											1

TABLE C2
Individual Animal Tumor Pathology of Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame: 6,250 ppm

Number of Days on Study	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
	1	1	7	7	7	7	7	7	7	7	7	7	7	7	7	
	4	0	4	4	4	4	4	4	4	4	4	5	5	5	5	
Carcass ID Number	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	Total
	4	4	4	4	4	4	4	5	5	5	5	4	4	5	5	Tissues/
	6	9	1	2	3	5	8	1	2	4	5	4	7	0	3	Tumors
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymphoma malignant	X	X														2

TABLE C2
Individual Animal Tumor Pathology of Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame: 12,500 ppm

Number of Days on Study	274	274	274	274	274	274	274	274	274	274	274	274	275	275	275	275	
Carcass ID Number	25057	25058	25059	26060	26061	26062	26064	26065	26066	26068	26070	27506	26063	26067	26069	26069	Total Tissues/ Tumors
Alimentary System																	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Cardiovascular System																	
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																	
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pituitary gland	+	M	I	I	I	+	I	I	+	+	+	M	+	+	+	+	8
Thyroid gland	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	14
General Body System																	
None																	
Genital System																	
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																	
Lymph node										+							1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	+	I	+	+	M	+	+	+	+	+	+	+	13
Lymph node, mediastinal	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	13
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	14
Integumentary System																	
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Musculoskeletal System																	
None																	
Nervous System																	
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Respiratory System																	
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Special Senses System																	
None																	

TABLE C2
Individual Animal Tumor Pathology of Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame: 12,500 ppm

Number of Days on Study	274	274	274	274	274	274	274	274	274	274	274	274	275	275	275	275	
Carcass ID Number	257	258	259	260	261	262	264	265	266	268	270	275	263	267	269		Total Tissues/ Tumors
Urinary System																	
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Systemic Lesions																	
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15

TABLE C2
Individual Animal Tumor Pathology of Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame: 25,000 ppm

Number of Days on Study	0 4 9	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	
Carcass ID Number	2 7 5	2 7 1	2 7 2	2 7 3	2 7 4	2 7 6	2 7 8	2 7 9	2 8 0	2 8 2	2 7 7	2 8 1	2 8 3	2 8 4	2 8 5	Total Tissues/ Tumors
Alimentary System																
Liver	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Salivary glands												+				1
Stomach, forestomach	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pituitary gland	A	+	M	+	I	+	I	+	+	+	+	+	+	I	+	10
Thyroid gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
General Body System																
None																
Genital System																
Epididymis	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Prostate	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Sarcoma							X									1
Testes	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Hematopoietic System																
Lymph node, mandibular	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mesenteric	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mediastinal	M	+	+	+	+	+	M	+	+	+	+	+	M	+	+	12
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Integumentary System																
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Musculoskeletal System																
None																
Nervous System																
Brain	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Respiratory System																
Lung	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Special Senses System																
None																

TABLE C2
Individual Animal Tumor Pathology of Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame: 25,000 ppm

Number of Days on Study	0 4 9	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	
Carcass ID Number	2 7 5	2 7 1	2 7 2	2 7 3	2 7 4	2 7 6	2 7 8	2 7 9	2 8 0	2 8 2	2 7 7	2 8 1	2 8 3	2 8 4	2 8 5	Total Tissues/ Tumors
Urinary System																
Kidney	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE C2
Individual Animal Tumor Pathology of Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame: 50,000 ppm

Number of Days on Study	0 7 1	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 5	2 7 5	2 7 5	
Carcass ID Number	2 9 1	2 8 6	2 8 7	2 8 9	2 9 2	2 9 4	2 9 5	2 9 6	2 9 7	2 9 8	2 9 9	3 0 0	2 8 8	2 9 0	2 9 3		Total Tissues/ Tumors
Alimentary System																	
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Gallbladder	+	+	+	+	+	+	+	M	+	M	+	+	M	+	+		12
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Polyp adenomatous			X														1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Tooth													+				1
Cardiovascular System																	
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Endocrine System																	
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Parathyroid gland	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+		13
Pituitary gland	I	+	+	+	+	+	+	+	+	M	+	+	+	I	+		12
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
General Body System																	
None																	
Genital System																	
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Preputial gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M		13
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Sarcoma									X								1
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15

TABLE C2
Individual Animal Tumor Pathology of Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame: 50,000 ppm

Number of Days on Study	0717	2744	2744	2744	2744	2744	2744	2744	2744	2744	2744	2744	2745	2745	2745	
Carcass ID Number	2911	2986	2987	2989	2992	2994	2995	2996	2997	2998	2999	3000	2988	2990	2993	Total Tissues/ Tumors
Hematopoietic System																
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mediastinal	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	13
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Integumentary System																
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Subcutaneous tissue, sarcoma	X															1
Musculoskeletal System																
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Nervous System																
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Respiratory System																
Larynx	M	M	+	M	M	M	M	I	M	+	M	M	M	M	I	2
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Special Senses System																
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Zymbal's gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	14
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	14
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE C3
Statistical Analysis of Primary Neoplasms in Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
All Organs: Malignant Lymphoma						
Overall rate[a]	0/15 (0%)	0/15 (0%)	2/15 (13%)	0/15 (0%)	0/15 (0%)	0/15 (0%)
Adjusted rate[b]	0.0%	0.0%	13.3%	0.0%	0.0%	0.0%
Terminal rate[c]	0/14 (0%)	0/15 (0%)	0/13 (0%)	0/15 (0%)	0/14 (0%)	0/14 (0%)
First incidence (days)	—[e]	—[f]	114	—	—	—
Poly-3 test[d]	P=0.378N	—	P=0.247	—	—	—
All Organs: Malignant Neoplasms						
Overall rate	0/15 (0%)	0/15 (0%)	2/15 (13%)	0/15 (0%)	1/15 (7%)	2/15 (13%)
Adjusted rate	0.0%	0.0%	13.3%	0.0%	7.1%	13.3%
Terminal rate	0/14 (0%)	0/15 (0%)	0/13 (0%)	0/15 (0%)	1/14 (7%)	1/14 (7%)
First incidence (days)	—	—	114	—	274 (T)	71
Poly-3 test	P=0.123	—	P=0.247	—	P=0.499	P=0.247
All Organs: Benign or Malignant Neoplasms						
Overall rate	0/15 (0%)	0/15 (0%)	2/15 (13%)	0/15 (0%)	1/15 (7%)	3/15 (20%)
Adjusted rate	0.0%	0.0%	13.3%	0.0%	7.1%	20.0%
Terminal rate	0/14 (0%)	0/15 (0%)	0/13 (0%)	0/15 (0%)	1/14 (7%)	2/14 (14%)
First incidence (days)	—	—	114	—	274 (T)	71
Poly-3 test	P=0.027	—	P=0.247	—	P=0.499	P=0.118

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

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame[a]

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Disposition Summary						
Animals initially in study	15	15	15	15	15	15
Early deaths						
Moribund			1			1
Natural deaths	1		1		1	
Survivors						
Terminal sacrifice	14	15	13	15	14	14
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Liver	(15)	(15)	(15)	(15)	(14)	(15)
Infiltration cellular, focal, lymphocyte	2 (13%)	2 (13%)	4 (27%)	1 (7%)	2 (14%)	5 (33%)
Inflammation, chronic	1 (7%)					
Inflammation, chronic active	3 (20%)					1 (7%)
Necrosis, focal	2 (13%)	1 (7%)				1 (7%)
Hepatocyte, periportal, vacuolization cytoplasmic, diffuse	2 (13%)					4 (27%)
Salivary glands	(15)	(1)	(1)		(1)	(15)
Infiltration cellular, focal, lymphocyte	9 (60%)	1 (100%)			1 (100%)	10 (67%)
Tooth						(1)
Peridontal tissue, cyst						1 (100%)
Cardiovascular System						
None						
Endocrine System						
Adrenal cortex	(15)	(15)	(15)	(15)	(15)	(15)
Atrophy	14 (93%)	14 (93%)	12 (80%)	12 (80%)	10 (67%)	13 (87%)
Hypertrophy, focal		1 (7%)	3 (20%)	2 (13%)	1 (7%)	3 (20%)
Subcapsular, hyperplasia, focal	1 (7%)		2 (13%)	1 (7%)	2 (13%)	1 (7%)
General Body System						
None						
Genital System						
Epididymis	(15)	(15)	(15)	(15)	(14)	(15)
Epithelium, degeneration, focal		1 (7%)				
Testes	(15)	(15)	(15)	(15)	(14)	(15)
Mineralization, focal		1 (7%)				
Germinal epithelium, degeneration, diffuse		1 (7%)				
Germinal epithelium, degeneration, focal		1 (7%)		1 (7%)		
Rete testes, inflammation, focal	1 (7%)					

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

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Hematopoietic System						
Bone marrow	(14)					(15)
Hyperplasia						1 (7%)
Lymph node				(1)		
Iliac, pigmentation, focal				1 (100%)		
Lymph node, mandibular	(14)	(15)	(14)	(15)	(14)	(15)
Hyperplasia		3 (20%)		2 (13%)		
Hyperplasia, plasma cell	1 (7%)					2 (13%)
Lymph node, mesenteric	(14)	(14)	(15)	(13)	(14)	(15)
Hyperplasia					1 (7%)	
Lymph node, mediastinal	(13)	(14)	(14)	(13)	(12)	(13)
Hyperplasia					1 (8%)	
Spleen	(15)	(15)	(15)	(15)	(14)	(15)
Atrophy	2 (13%)					
Hematopoietic cell proliferation					1 (7%)	2 (13%)
Hyperplasia, lymphoid						1 (7%)
Thymus	(14)	(15)	(15)	(14)	(15)	(14)
Atrophy, diffuse					1 (7%)	
Atrophy, focal						2 (14%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
Brain	(14)	(15)	(15)	(15)	(14)	(15)
Hydrocephalus			1 (7%)			
Corpus callosum, medulla, vacuolization cytoplasmic, focal			1 (7%)			
Medulla, vacuolization cytoplasmic, focal						1 (7%)
Ventricle, hydrocephalus		1 (7%)				
Peripheral nerve	(1)					
Vacuolization cytoplasmic, diffuse	1 (100%)					
Spinal cord	(1)					
Vacuolization cytoplasmic, focal	1 (100%)					
Respiratory System						
Lung	(15)	(15)	(15)	(15)	(14)	(15)
Infiltration cellular, focal, lymphocyte						1 (7%)
Inflammation, chronic active, diffuse			1 (7%)			
Perivascular, infiltration cellular, lymphocyte					2 (14%)	
Nose	(14)					(15)
Inflammation, acute	1 (7%)					

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Special Senses System						
Eye	(14)		(1)			(15)
Developmental malformation			1 (100%)			
Harderian gland	(15)					(15)
Infiltration cellular, focal, lymphocyte	5 (33%)					1 (7%)
Inflammation, chronic active						1 (7%)
Urinary System						
Kidney	(14)	(15)	(15)	(15)	(14)	(15)
Infiltration cellular, focal, lymphocyte		1 (7%)		1 (7%)		
Nephropathy	4 (29%)					3 (20%)
Renal tubule, dilatation, diffuse		2 (13%)		1 (7%)		1 (7%)
Renal tubule, dilatation, focal		2 (13%)				

APPENDIX D
SUMMARY OF LESIONS
IN FEMALE p53 HAPLOINSUFFICIENT MICE
IN THE 9-MONTH FEED STUDY
OF ASPARTAME

TABLE D1
Summary of the Incidence of Neoplasms in Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame[a]

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Disposition Summary						
Animals initially in study	15	15	15	15	15	15
Early deaths						
Moribund	1		1			
Natural deaths		1				
Survivors						
Terminal sacrifice	14	14	14	15	15	15
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Gallbladder	(14)					(15)
Intestine large, cecum	(15)				(1)	(15)
Liver	(15)	(15)	(15)	(15)	(15)	(15)
Sarcoma				1 (7%)		
Cardiovascular System						
None						
Endocrine System						
Pituitary gland	(9)	(14)	(13)	(14)	(11)	(14)
Pars distalis, adenoma	1 (11%)					
Thyroid gland	(14)	(13)	(14)	(14)	(13)	(15)
Follicular cell, adenoma					1 (8%)	
General Body System						
None						
Genital System						
None						
Hematopoietic System						
Lymph node					(1)	
Lymph node, mandibular	(15)	(13)	(13)	(15)	(15)	(14)
Lymph node, mesenteric	(15)	(14)	(14)	(14)	(15)	(15)
Sarcoma				1 (7%)		
Lymph node, mediastinal	(12)	(13)	(13)	(12)	(13)	(15)
Spleen	(15)	(15)	(15)	(15)	(15)	(15)
Thymus	(14)	(14)	(14)	(15)	(15)	(15)
Integumentary System						
Mammary gland	(13)	(14)	(14)	(15)	(12)	(15)
Carcinoma	1 (8%)			1 (7%)		
Skin	(15)		(1)			(15)
Subcutaneous tissue, fibrosarcoma			1 (100%)			

TABLE D1
Summary of the Incidence of Neoplasms in Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Musculoskeletal System						
Bone	(15)		(1)			(15)
Femur, osteosarcoma	1 (7%)					1 (7%)
Vertebra, osteosarcoma			1 (100%)			
Nervous System						
None						
Respiratory System						
Lung	(15)	(15)	(14)	(15)	(15)	(15)
Special Senses System						
None						
Urinary System						
Kidney	(15)	(14)	(15)	(15)	(15)	(15)
Systemic Lesions						
Multiple organs[b]	(15)	(15)	(15)	(15)	(15)	(15)
Lymphoma malignant	1 (7%)				1 (7%)	
Neoplasm Summary						
Total animals with primary neoplasms[c]	4		2	2	1	1
Total primary neoplasms	4		2	3	2	1
Total animals with benign neoplasms	1				1	
Total benign neoplasms	1				1	
Total animals with malignant neoplasms	3		2	2	1	1
Total malignant neoplasms	3		2	3	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 D2
Individual Animal Tumor Pathology of Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame: 0 ppm

Number of Days on Study	0 3 6	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	
Carcass ID Number	3 1 3	3 0 4	3 0 7	3 0 8	3 1 0	3 1 1	3 0 1	3 0 2	3 0 3	3 0 5	3 0 6	3 0 9	3 1 2	3 1 4	3 1 5	Total Tissues/ Tumors
Alimentary System																
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Gallbladder	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Cardiovascular System																
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Parathyroid gland	M	M	M	+	+	+	M	M	M	+	M	M	M	+	+	6
Pituitary gland	I	+	+	+	+	+	M	M	+	I	+	I	+	I	+	9
Pars distalis, adenoma						X										1
Thyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	14
General Body System																
None																
Genital System																
Clitoral gland	M	+	+	+	+	+	M	+	+	+	+	+	+	+	M	12
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

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

TABLE D2
Individual Animal Tumor Pathology of Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame: 0 ppm

Number of Days on Study	0 3 6	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	
Carcass ID Number	3 1 3	3 0 4	3 0 7	3 0 8	3 1 0	3 1 1	3 0 1	3 0 2	3 0 3	3 0 5	3 0 6	3 0 9	3 1 2	3 1 4	3 1 5	Total Tissues/ Tumors
Hematopoietic System																
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mediastinal	+	+	M	+	+	+	M	+	+	+	+	+	+	M	+	12
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	14
Integumentary System																
Mammary gland	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	13
Carcinoma							X									1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Musculoskeletal System																
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Femur, osteosarcoma										X						1
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Nervous System																
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Respiratory System																
Larynx	+	+	M	+	+	I	M	M	M	M	M	M	I	M	M	4
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Special Senses System																
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Zymbal's gland	+	M	+	+	+	+	M	M	+	+	+	+	+	+	+	12
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Urinary bladder	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymphoma malignant			X													1

TABLE D2
Individual Animal Tumor Pathology of Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame: 3,125 ppm

Number of Days on Study	0 9 8	2 7 5	2 7 5	2 7 5	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	
Carcass ID Number	3 2 5	3 1 7	3 1 9	3 2 6	3 1 6	3 1 8	3 2 0	3 2 1	3 2 2	3 2 3	3 2 4	3 2 7	3 2 8	3 2 9	3 3 0		Total Tissues/ Tumors
Alimentary System																	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Cardiovascular System																	
Blood vessel					+												1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Endocrine System																	
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Thyroid gland	A	M	+	+	+	+	+	+	+	+	+	+	+	+	+		13
General Body System																	
None																	
Genital System																	
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Hematopoietic System																	
Lymph node, mandibular	A	+	+	+	+	+	+	+	+	+	+	+	+	M	+		13
Lymph node, mesenteric	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Lymph node, mediastinal	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+		13
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Thymus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Integumentary System																	
Mammary gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Musculoskeletal System																	
None																	
Nervous System																	
Brain	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Respiratory System																	
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Special Senses System																	
None																	

TABLE D2
Individual Animal Tumor Pathology of Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame: 3,125 ppm

Number of Days on Study	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
	9	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
	8	5	5	5	8	8	8	8	8	8	8	8	8	8	8	
Carcass ID Number	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	Total
	2	1	1	2	1	1	2	2	2	2	2	2	2	2	3	Tissues/
	5	7	9	6	6	8	0	1	2	3	4	7	8	9	0	Tumors
Urinary System																
Kidney	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE D2
Individual Animal Tumor Pathology of Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame: 6,250 ppm

Number of Days on Study	1 6 8	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	
Carcass ID Number	3 4 1	3 3 2	3 3 5	3 3 6	3 3 9	3 4 0	3 4 3	3 3 1	3 3 3	3 3 4	3 3 7	3 3 8	3 4 2	3 4 4	3 4 5	Total Tissues/ Tumors
Alimentary System																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Stomach, glandular	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Cardiovascular System																
Heart	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Endocrine System																
Adrenal cortex	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Adrenal medulla	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Pituitary gland	M	+	+	+	I	+	+	+	+	+	+	+	+	+	+	13
Thyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	14
General Body System																
None																
Genital System																
Ovary	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Lymph node, mandibular	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	13
Lymph node, mesenteric	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mediastinal	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	13
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Integumentary System																
Mammary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Skin					+											1
Subcutaneous tissue, fibrosarcoma					X											1
Musculoskeletal System																
Bone						+										1
Vertebra, osteosarcoma						X										1
Nervous System																
Brain	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Respiratory System																
Lung	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Special Senses System																
None																

TABLE D2
Individual Animal Tumor Pathology of Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame: 6,250 ppm

Number of Days on Study	1 6 8	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	2 7 8	
Carcass ID Number	3 4 1	3 3 2	3 3 5	3 3 6	3 3 9	3 4 0	3 4 3	3 3 1	3 3 3	3 3 4	3 3 7	3 3 8	3 4 2	3 4 4	3 4 5	Total Tissues/ Tumors
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE D2
Individual Animal Tumor Pathology of Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame: 12,500 ppm

Number of Days on Study	27575	27755	27755	27755	27755	27755	27755	27755	27755	27758	27778	27778	27778	27778	27778	
Carcass ID Number	34746	34749	35500	35553	35554	35555	35557	35559	36560	34047	34478	35011	35552	35568	35558	Total Tissues/ Tumors
Alimentary System																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Sarcoma							X									1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pituitary gland	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	14
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	14
General Body System																
None																
Genital System																
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	14
Sarcoma							X									1
Lymph node, mediastinal	+	+	+	+	+	M	M	+	+	M	+	+	+	+	+	12
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Integumentary System																
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Carcinoma			X													1
Musculoskeletal System																
None																
Nervous System																
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Respiratory System																
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Special Senses System																
None																

TABLE D2
Individual Animal Tumor Pathology of Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame: 12,500 ppm

Number of Days on Study	275	275	275	275	275	275	275	275	275	278	278	278	278	278	278	278	
Carcass ID Number	34696	34949	35050	35533	35544	35555	35575	35595	35606	34707	34948	35051	35542	35553	35566	35588	Total Tissues/ Tumors
Urinary System																	
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Systemic Lesions																	
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE D2
Individual Animal Tumor Pathology of Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame: 25,000 ppm

Number of Days on Study	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	
	75	75	75	75	75	75	78	78	78	78	78	78	78	78	78	78	
Carcass ID Number	36	36	36	36	37	37	36	36	36	36	36	37	37	37	37	37	Total Tissues/ Tumors
	11	14	17	19	14	15	12	13	15	16	18	10	11	12	13	14	
Alimentary System																	
Intestine large, cecum													+				1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Cardiovascular System																	
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																	
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pituitary gland	+	+	+	M	+	+	I	+	+	M	+	+	I	+	+		11
Thyroid gland	+	+	+	+	+	+	I	+	+	+	+	M	+	+	+		13
Follicular cell, adenoma													X				1
General Body System																	
None																	
Genital System																	
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																	
Lymph node													+				1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mediastinal	+	+	+	M	+	+	+	+	+	I	+	+	+	+	+	+	13
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Integumentary System																	
Mammary gland	+	+	+	M	+	+	+	+	+	+	+	+	M	+	M		12
Musculoskeletal System																	
None																	
Nervous System																	
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Respiratory System																	
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Special Senses System																	
None																	

TABLE D2
Individual Animal Tumor Pathology of Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame: 25,000 ppm

Number of Days on Study	275	275	275	275	275	275	278	278	278	278	278	278	278	278	278	
Carcass ID Number	36 1	36 4	36 7	36 9	37 4	37 5	36 2	36 3	36 5	36 6	36 8	37 0	37 1	37 2	37 3	Total Tissues/ Tumors
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymphoma malignant													X			1

TABLE D2
Individual Animal Tumor Pathology of Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame: 50,000 ppm

Number of Days on Study	275	275	275	275	275	275	278	278	278	278	278	278	278	278	278	
Carcass ID Number	37616	37618	38803	38804	38805	38808	37807	37809	38800	38801	38802	38806	38807	38809	38900	Total Tissues/ Tumors
Alimentary System																
Esophagus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	14
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Cardiovascular System																
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Parathyroid gland	+	+	+	M	+	M	+	+	+	+	M	+	+	M	+	11
Pituitary gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	14
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
None																
Genital System																
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	14
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mediastinal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE D2
Individual Animal Tumor Pathology of Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame: 50,000 ppm

Number of Days on Study	275	275	275	275	275	275	278	278	278	278	278	278	278	278	278	
Carcass ID Number	37676	37688	38303	38804	38805	38808	37707	37709	38800	38801	38802	38806	38807	38809	38900	Total Tissues/ Tumors
Integumentary System																
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Musculoskeletal System																
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Femur, osteosarcoma											X					1
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Nervous System																
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Respiratory System																
Larynx	I	M	I	I	+	I	+	M	+	+	I	I	I	+	I	5
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Special Senses System																
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Zymbal's gland	+	+	+	+	M	M	+	+	+	+	M	+	+	M	+	11
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE D3
Statistical Analysis of Primary Neoplasms in Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
All Organs: Malignant Lymphoma						
Overall rate[a]	1/15 (7%)	0/15 (0%)	0/15 (0%)	0/15 (0%)	1/15 (7%)	0/15 (0%)
Adjusted rate[b]	7.1%	0.0%	0.0%	0.0%	6.7%	0.0%
Terminal rate[c]	1/14 (7%)	0/14 (0%)	0/14 (0%)	0/15 (0%)	1/15 (7%)	0/15 (0%)
First incidence (days)	275 (T)	—[e]	—	—	275 (T)	—
Poly-3 test[d]	P=0.578N	P=0.499N	P=0.497N	P=0.486N	P=0.745N	P=0.486N
All Organs: Malignant Neoplasms						
Overall rate	3/15 (20%)	0/15 (0%)	2/15 (13%)	2/15 (13%)	1/15 (7%)	1/15 (7%)
Adjusted rate	21.4%	0.0%	14.1%	13.3%	6.7%	6.7%
Terminal rate	3/14 (21%)	0/14 (0%)	2/14 (14%)	2/15 (13%)	1/15 (7%)	1/15 (7%)
First incidence (days)	275 (T)	—	275 (T)	275 (T)	275 (T)	275 (T)
Poly-3 test	P=0.315N	P=0.105N	P=0.492N	P=0.467N	P=0.273N	P=0.273N
All Organs: Benign or Malignant Neoplasms						
Overall rate	4/15 (27%)	0/15 (0%)	2/15 (13%)	2/15 (13%)	1/15 (7%)	1/15 (7%)
Adjusted rate	28.6%	0.0%	14.1%	13.3%	6.7%	6.7%
Terminal rate	4/14 (29%)	0/14 (0%)	2/14 (14%)	2/15 (13%)	1/15 (7%)	1/15 (7%)
First incidence (days)	275 (T)	—	275 (T)	275 (T)	275 (T)	275 (T)
Poly-3 test	P=0.217N	P=0.044N	P=0.318N	P=0.293N	P=0.141N	P=0.141N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame[a]

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Disposition Summary						
Animals initially in study	15	15	15	15	15	15
Early deaths						
Moribund	1		1			
Natural deaths		1				
Survivors						
Terminal sacrifice	14	14	14	15	15	15
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Liver	(15)	(15)	(15)	(15)	(15)	(15)
Infiltration cellular, focal, lymphocyte	4 (27%)	3 (20%)	4 (27%)	6 (40%)	4 (27%)	4 (27%)
Necrosis, focal						1 (7%)
Pigmentation, focal, hemosiderin	1 (7%)					
Tension lipidosis	1 (7%)					
Hepatocyte, necrosis, focal					2 (13%)	
Hepatocyte, vacuolization cytoplasmic, focal					1 (7%)	
Salivary glands	(15)					(15)
Infiltration cellular, focal, lymphocyte	6 (40%)					8 (53%)
Cardiovascular System						
None						
Endocrine System						
Adrenal cortex	(15)	(15)	(14)	(15)	(15)	(15)
Hyperplasia, focal			1 (7%)	1 (7%)	1 (7%)	
Subcapsular, hyperplasia, focal	12 (80%)	13 (87%)	13 (93%)	15 (100%)	15 (100%)	15 (100%)
Pituitary gland	(9)	(14)	(13)	(14)	(11)	(14)
Pars intermedia, hypertrophy			1 (8%)		2 (18%)	2 (14%)
Thyroid gland	(14)	(13)	(14)	(14)	(13)	(15)
Ectopic thymus	2 (14%)	1 (8%)	1 (7%)	3 (21%)	1 (8%)	2 (13%)
General Body System						
None						
Genital System						
Ovary	(15)	(15)	(14)	(15)	(15)	(15)
Atrophy			1 (7%)			
Cyst	1 (7%)					
Uterus	(15)	(15)	(15)	(15)	(15)	(15)
Hydrometra		1 (7%)				
Endometrium, hyperplasia, cystic	14 (93%)	14 (93%)	15 (100%)	14 (93%)	15 (100%)	15 (100%)

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

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Hematopoietic System						
Lymph node, mandibular	(15)	(13)	(13)	(15)	(15)	(14)
Congestion					1 (7%)	
Hyperplasia	1 (7%)	2 (15%)		1 (7%)	1 (7%)	
Hyperplasia, histiocytic	1 (7%)					
Hyperplasia, lymphoid	2 (13%)					
Hyperplasia, plasma cell	1 (7%)					
Lymph node, mesenteric	(15)	(14)	(14)	(14)	(15)	(15)
Hyperplasia		1 (7%)				
Hyperplasia, lymphoid	1 (7%)					
Lymph node, mediastinal	(12)	(13)	(13)	(12)	(13)	(15)
Hyperplasia, lymphoid	1 (8%)					
Spleen	(15)	(15)	(15)	(15)	(15)	(15)
Depletion cellular, diffuse		1 (7%)				
Hematopoietic cell proliferation	3 (20%)	4 (27%)	1 (7%)	3 (20%)	1 (7%)	1 (7%)
Pigmentation					1 (7%)	
Thymus	(14)	(14)	(14)	(15)	(15)	(15)
Atrophy, focal	1 (7%)					
Hyperplasia			1 (7%)			1 (7%)
Hyperplasia, atypical, focal				1 (7%)		
Integumentary System						
None						
Musculoskeletal System						
Bone	(15)		(1)			(15)
Femur, fibrous osteodystrophy, focal						1 (7%)
Nervous System						
Brain	(15)	(14)	(14)	(15)	(15)	(15)
Medulla, cyst epithelial inclusion				1 (7%)		
Respiratory System						
Lung	(15)	(15)	(14)	(15)	(15)	(15)
Infiltration cellular, focal, lymphocyte	1 (7%)				1 (7%)	1 (7%)
Special Senses System						
Harderian gland	(15)					(15)
Infiltration cellular, focal, lymphocyte	1 (7%)					3 (20%)
Inflammation, chronic active						1 (7%)
Urinary System						
Kidney	(15)	(14)	(15)	(15)	(15)	(15)
Infiltration cellular, focal, lymphocyte		1 (7%)		1 (7%)		
Glomerulus, hyalinization						1 (7%)
Renal tubule, dilatation, diffuse	1 (7%)	2 (14%)	3 (20%)			
Renal tubule, dilatation, focal	6 (40%)	5 (36%)	2 (13%)	5 (33%)	4 (27%)	4 (27%)

APPENDIX E
SUMMARY OF LESIONS
IN MALE Cdkn2a DEFICIENT MICE
IN THE 9-MONTH FEED STUDY
OF ASPARTAME

TABLE E1
Summary of the Incidence of Neoplasms in Male Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame[a]

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Disposition Summary						
Animals initially in study	15	15	15	15	15	15
Early deaths						
Natural death	1	1		1	1	
Survivors						
Terminal sacrifice	14	14	15	14	14	15
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Liver	(15)	(15)	(15)	(14)	(15)	(15)
Histiocytic sarcoma		1 (7%)				
Cardiovascular System						
Heart	(15)	(15)	(15)	(15)	(15)	(15)
Histiocytic sarcoma		1 (7%)				
Endocrine System						
None						
General Body System						
None						
Genital System						
None						
Hematopoietic System						
Bone marrow	(15)					(15)
Histiocytic sarcoma	2 (13%)					
Lymph node, mandibular	(15)	(15)	(15)	(14)	(14)	(15)
Histiocytic sarcoma		1 (7%)				
Lymph node, mesenteric	(15)	(15)	(15)	(14)	(14)	(15)
Histiocytic sarcoma		1 (7%)				
Spleen	(15)	(15)	(15	(14)	(15)	(15)
Hemangiosarcoma		1 (7%)				
Histiocytic sarcoma		1 (7%)				
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						

TABLE E1
Summary of the Incidence of Neoplasms in Male Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Respiratory System						
Lung	(15)	(15)	(15)	(15)	(15)	(15)
Alveolar/bronchiolar adenoma				1 (7%)		1 (7%)
Histiocytic sarcoma		1 (7%)				
Special Senses System						
None						
Urinary System						
Kidney	(15)	(15)	(15)	(14)	(15)	(15)
Histiocytic sarcoma		1 (7%)				
Systemic Lesions						
Multiple organs[b]	(15)	(15)	(15)	(15)	(15)	(15)
Histiocytic sarcoma	2 (13%)	1 (7%)				
Neoplasm Summary						
Total animals with primary neoplasms[c]	2	2		1		1
Total primary neoplasms	2	2		1		1
Total animals with benign neoplasms				1		1
Total benign neoplasms				1		1
Total animals with malignant neoplasms	2	2				
Total malignant neoplasms	2	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 E2
Individual Animal Tumor Pathology of Male Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame: 0 ppm

Number of Days on Study	0 6 3	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 5	2 7 5	
Carcass ID Number	4 0 1	3 9 1	3 9 2	3 9 3	3 9 4	3 9 5	3 9 7	3 9 8	3 9 9	4 0 0	4 0 2	4 0 4	4 0 5	3 9 6	4 0 3		Total Tissues/ Tumors
Alimentary System																	
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I		14
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Intestine large, rectum	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+		14
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Intestine small, jejunum	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+		13
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Cardiovascular System																	
Blood vessel	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+		14
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Endocrine System																	
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Islets, pancreatic	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+		14
Parathyroid gland	M	M	+	+	M	M	+	+	+	+	+	M	+	M	M		8
Pituitary gland	+	+	+	I	+	+	+	+	+	+	+	+	+	+	I		13
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+		14
General Body System																	
None																	
Genital System																	
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Preputial gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+		14
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15

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

TABLE E2
Individual Animal Tumor Pathology of Male Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame: 0 ppm

Number of Days on Study	0 6 3	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 5	2 7 5	
Carcass ID Number	4 0 1	3 9 1	3 9 2	3 9 3	3 9 4	3 9 5	3 9 7	3 9 8	3 9 9	4 0 0	4 0 2	4 0 4	4 0 5	3 9 6	4 0 3		Total Tissues/ Tumors
Hematopoietic System																	
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Histiocytic sarcoma						X					X						2
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Lymph node, mediastinal	M	+	+	+	+	+	+	M	+	+	M	+	+	+	+		12
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Integumentary System																	
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M		
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Musculoskeletal System																	
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Nervous System																	
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Respiratory System																	
Larynx	I	+	M	M	M	M	M	M	M	M	M	+	M	M	M		2
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Trachea	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+		14
Special Senses System																	
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Zymbal's gland	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+		13
Urinary System																	
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Systemic Lesions																	
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Histiocytic sarcoma						X					X						2

TABLE E2
Individual Animal Tumor Pathology of Male Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame: 3,125 ppm

Number of Days on Study	232	274	274	274	274	274	274	274	274	274	275	275	275	275	275	275	
Carcass ID Number	40006	40008	40009	40014	40015	40016	40017	40018	40020	40007	40010	40011	40012	40013	40019		Total Tissues/ Tumors
Alimentary System																	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Histiocytic sarcoma	X																1
Stomach, forestomach	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Cardiovascular System																	
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Histiocytic sarcoma	X																1
Endocrine System																	
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Pituitary gland	+	I	+	+	I	+	+	+	I	+	+	+	I	I	+		10
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
General Body System																	
None																	
Genital System																	
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Hematopoietic System																	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Histiocytic sarcoma	X																1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Histiocytic sarcoma	X																1
Lymph node, mediastinal	M	+	+	+	I	+	+	+	+	+	+	+	+	+	+		13
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Hemangiosarcoma				X													1
Histiocytic sarcoma	X																1
Thymus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Integumentary System																	
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Musculoskeletal System																	
Skeletal muscle											+						1
Nervous System																	
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15

TABLE E2
Individual Animal Tumor Pathology of Male Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame: 3,125 ppm

Number of Days on Study	223	274	274	274	274	274	274	274	274	275	275	275	275	275	275	
Carcass ID Number	40006	40008	40009	40014	40015	40016	40017	40018	40020	40107	40110	40111	40112	40113	40119	Total Tissues/ Tumors
Respiratory System																
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma	X															1
Special Senses System																
None																
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma	X															1
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma	X															1

TABLE E2
Individual Animal Tumor Pathology of Male Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame: 6,250 ppm

Number of Days on Study	274	274	274	274	274	274	274	274	274	275	275	275	275	275	275	
Carcass ID Number	42242	42244	42246	42247	42249	43231	43232	43233	43235	42241	42243	42245	42248	43230	43234	Total Tissues/ Tumors
Alimentary System																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pituitary gland	I	I	I	I	+	+	+	I	+	+	+	+	+	+	+	10
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
None																
Genital System																
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mediastinal	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	13
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Integumentary System																
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Musculoskeletal System																
None																
Nervous System																
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Respiratory System																
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Special Senses System																
None																

TABLE E2
Individual Animal Tumor Pathology of Male Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame: 6,250 ppm

Number of Days on Study	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	
Carcass ID Number	4 2 2	4 2 4	4 2 6	4 2 7	4 2 9	4 3 1	4 3 2	4 3 3	4 3 5	4 2 1	4 2 3	4 2 5	4 2 8	4 3 0	4 3 4	Total Tissues/ Tumors
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE E2
Individual Animal Tumor Pathology of Male Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame: 12,500 ppm

Number of Days on Study	0 4 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 5	2 7 5	2 7 5	2 7 5	
Carcass ID Number	4 3 6	4 3 8	4 3 9	4 4 0	4 4 2	4 4 3	4 4 5	4 4 6	4 4 8	4 4 9	4 5 0	4 3 7	4 4 1	4 4 4	4 4 7	Total Tissues/ Tumors
Alimentary System																
Liver	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Stomach, forestomach	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Adrenal medulla	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Pituitary gland	A	+	+	+	+	+	+	+	+	+	+	+	+	I	I	12
Thyroid gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
General Body System																
None																
Genital System																
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Testes	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Hematopoietic System																
Lymph node, mandibular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mesenteric	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mediastinal	A	+	+	+	+	+	+	+	+	M	+	+	+	+	+	13
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Thymus	A	+	+	+	+	+	+	+	+	+	+	M	+	+	+	13
Integumentary System																
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Musculoskeletal System																
None																
Nervous System																
Brain	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Respiratory System																
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Alveolar/bronchiolar adenoma												X				1
Special Senses System																
None																

TABLE E2
Individual Animal Tumor Pathology of Male Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame: 12,500 ppm

Number of Days on Study	0 4 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 5	2 7 5	2 7 5	2 7 5	
Carcass ID Number	4 3 6	4 3 8	4 3 9	4 4 0	4 4 2	4 4 3	4 4 5	4 4 6	4 4 8	4 4 9	4 4 0	4 5 7	4 3 1	4 4 4	4 4 7	Total Tissues/ Tumors
Urinary System																
Kidney	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE E2
Individual Animal Tumor Pathology of Male Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame: 25,000 ppm

Number of Days on Study	0 2 2	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 5	2 7 5	
Carcass ID Number	4 6 5	4 5 1	4 5 2	4 5 3	4 5 4	4 5 5	4 5 7	4 5 8	4 5 9	4 6 0	4 6 1	4 6 3	4 6 4	4 6 6	4 5 6	4 6 2	Total Tissues/ Tumors
Alimentary System																	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Cardiovascular System																	
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																	
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pituitary gland	I	+	I	I	+	I	+	I	+	+	+	+	+	+	+	+	10
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																	
None																	
Genital System																	
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Prostate	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	13
Testes	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Hematopoietic System																	
Lymph node, mandibular	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mesenteric	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mediastinal	+	+	+	+	+	I	+	+	+	+	+	M	+	+	+	+	13
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Integumentary System																	
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Musculoskeletal System																	
None																	
Nervous System																	
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Respiratory System																	
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Special Senses System																	
None																	

TABLE E2
Individual Animal Tumor Pathology of Male Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame: 25,000 ppm

Number of Days on Study	0272	2744	2774	2774	2774	2774	2774	2774	2774	2774	2774	2774	2774	2775	2775	
Carcass ID Number	4665	4551	4552	4553	4554	4555	4557	4558	4559	4560	4661	4663	4664	4656	4662	Total Tissues/ Tumors
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE E2
Individual Animal Tumor Pathology of Male Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame: 50,000 ppm

Number of Days on Study	27744	27744	27744	27744	27744	27744	27744	27744	27744	27745	27745	27745	27745	27745	27745	
Carcass ID Number	46767	46769	47700	47701	47702	47705	47708	47709	48000	46606	46608	47703	47704	47706	47707	Total Tissues/ Tumors
Alimentary System																
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine large, rectum	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	13
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Cardiovascular System																
Blood vessel	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Parathyroid gland	+	M	M	+	M	+	M	+	+	M	+	M	+	M	+	8
Pituitary gland	+	+	+	I	+	+	+	I	I	+	+	I	+	+	+	11
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
None																
Genital System																
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Preputial gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	14
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mediastinal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE E2
Individual Animal Tumor Pathology of Male Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame: 50,000 ppm

Number of Days on Study	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	
Carcass ID Number	4 6 7	4 6 9	4 7 0	4 7 1	4 7 2	4 7 5	4 7 8	4 7 9	4 8 0	4 6 6	4 6 8	4 7 3	4 7 4	4 7 6	4 7 7	Total Tissues/ Tumors
Integumentary System																
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Musculoskeletal System																
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Nervous System																
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Respiratory System																
Larynx	+	M	M	+	M	M	I	M	+	I	I	M	M	M	I	3
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Alveolar/bronchiolar adenoma		X														1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Special Senses System																
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Zymbal's gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	14
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE E3
Statistical Analysis of Primary Neoplasms in Male Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
All Organs: Hemangiosarcoma						
Overall rate[a]	0/15 (0%)	1/15 (7%)	0/15 (0%)	0/15 (0%)	0/15 (0%)	0/15 (0%)
Adjusted rate[b]	0.0%	6.9%	0.0%	0.0%	0.0%	0.0%
Terminal rate[c]	0/14 (0%)	1/14 (7%)	0/15 (0%)	0/14 (0%)	0/14 (0%)	0/15 (0%)
First incidence (days)	—[e]	274 (T)	—[f]	—	—	—
Poly-3 test[d]	P=0.494N	P=0.508	—	—	—	—
All Organs: Histiocytic Sarcoma						
Overall rate	2/15 (13%)	1/15 (7%)	0/15 (0%)	0/15 (0%)	0/15 (0%)	0/15 (0%)
Adjusted rate	14.3%	6.7%	0.0%	0.0%	0.0%	0.0%
Terminal rate	2/14 (14%)	0/14 (0%)	0/15 (0%)	0/14 (0%)	0/14 (0%)	0/15 (0%)
First incidence (days)	274 (T)	232	—	—	—	—
Poly-3 test	P=0.138N	P=0.476N	P=0.216N	P=0.231N	P=0.231N	P=0.216N
All Organs: Malignant Tumors						
Overall rate	2/15 (13%)	2/15 (13%)	0/15 (0%)	0/15 (0%)	0/15 (0%)	0/15 (0%)
Adjusted rate	14.3%	13.3%	0.0%	0.0%	0.0%	0.0%
Terminal rate	2/14 (14%)	1/14 (7%)	0/15 (0%)	0/14 (0%)	0/14 (0%)	0/15 (0%)
First incidence (days)	274 (T)	232	—	—	—	—
Poly-3 test	P=0.087N	P=0.673N	P=0.216N	P=0.231N	P=0.231N	P=0.216N
Organs: Benign or Malignant Neoplasms						
Overall rate	2/15 (13%)	2/15 (13%)	0/15 (0%)	1/15 (7%)	0/15 (0%)	1/15 (7%)
Adjusted rate	14.3%	13.3%	0.0%	7.1%	0.0%	6.7%
Terminal rate	2/14 (14%)	1/14 (7%)	0/15 (0%)	1/14 (7%)	0/14 (0%)	1/15 (7%)
Incidence (days)	274 (T)	232	—	274 (T)	—	274 (T)
Poly-3 test	P=0.361N	P=0.673N	P=0.216N	P=0.500N	P=0.231N	P=0.476

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

TABLE E4
Summary of the Incidence of Noneoplastic Lesions in Male Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame[a]

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Disposition Summary						
Animals initially in study	15	15	15	15	15	15
Early deaths						
Natural death	1	1		1	1	
Survivors						
Terminal sacrifice	14	14	15	14	14	15
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Liver	(15)	(15)	(15)	(14)	(15)	(15)
Infiltration cellular, focal, lymphocyte	3 (20%)					1 (7%)
Inflammation, acute	2 (13%)					
Necrosis, focal	2 (13%)		3 (20%)		1 (7%)	4 (27%)
Hepatocyte, necrosis, focal		1 (7%)	1 (7%)		1 (7%)	1 (7%)
Hepatocyte, vacuolization cytoplasmic, diffuse				1 (7%)		
Hepatocyte, periportal, vacuolization cytoplasmic	6 (40%)	11 (73%)	14 (93%)	8 (57%)	13 (87%)	13 (87%)
Salivary glands	(15)					(15)
Infiltration cellular, focal, lymphocyte	12 (80%)					11 (73%)
Cardiovascular System						
None						
Endocrine System						
Adrenal cortex	(15)	(15)	(15)	(14)	(15)	(15)
Accessory adrenal cortical nodule						1 (7%)
Subcapsular, hyperplasia, focal	2 (13%)	1 (7%)			1 (7%)	4 (27%)
General Body System						
None						
Genital System						
Epididymis	(15)	(15)	(15)	(15)	(15)	(15)
Inflammation, chronic active, focal			1 (7%)			
Bilateral, spermatozoa, degeneration	1 (7%)					
Testes	(15)	(15)	(15)	(14)	(14)	(15)
Germinal epithelium, degeneration		1 (7%)	1 (7%)			

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

TABLE E4
Summary of the Incidence of Noneoplastic Lesions in Male Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Hematopoietic System						
Lymph node, mandibular	(15)	(15)	(15)	(14)	(14)	(15)
Hyperplasia	1 (7%)			1 (7%)		
Necrosis	1 (7%)					
Lymph node, mesenteric	(15)	(15)	(15)	(14)	(14)	(15)
Hyperplasia			2 (13%)			
Spleen	(15)	(15)	(15)	(14)	(15)	(15)
Atrophy, diffuse	1 (7%)				1 (7%)	
Hematopoietic cell proliferation		1 (7%)	2 (13%)	3 (21%)		1 (7%)
Pigmentation						1 (7%)
Lymphoid follicle, atrophy	1 (7%)					
Thymus	(15)	(14)	(15)	(13)	(15)	(15)
Atrophy, diffuse	1 (7%)		1 (7%)		1 (7%)	
Atrophy, focal	1 (7%)	2 (14%)			1 (7%)	
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
Brain	(15)	(15)	(15)	(14)	(15)	(15)
Cerebellum, medulla, vacuolization cytoplasmic	1 (7%)	1 (7%)				
Respiratory System						
Lung	(15)	(15)	(15)	(15)	(15)	(15)
Infiltration cellular, focal, lymphocyte		1 (7%)				
Special Senses System						
Harderian gland	(15)					(15)
Infiltration cellular, lymphocyte	2 (13%)					2 (13%)
Urinary System						
Kidney	(15)	(15)	(15)	(14)	(15)	(15)
Nephropathy	2 (13%)					7 (47%)

APPENDIX F
SUMMARY OF LESIONS
IN FEMALE Cdkn2a DEFICIENT MICE
IN THE 9-MONTH FEED STUDY
OF ASPARTAME

TABLE F1
Summary of the Incidence of Neoplasms in Female Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame[a]

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Disposition Summary						
Animals initially in study	15	15	15	15	15	15
Early deaths						
Moribund sacrifice						1
Natural death	2		2			
Survivors						
Terminal sacrifice	13	15	13	15	15	14
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Gallbladder	(12)	(1)				(14)
Histiocytic sarcoma		1 (100%)				
Intestine large, cecum	(13)		(1)			(15)
Leiomyoma			1 (100%)			
Liver	(15)	(15)	(15)	(15)	(15)	(15)
Histiocytic sarcoma	2 (13%)	1 (7%)	1 (7%)			2 (13%)
Pancreas	(15)	(1)				(15)
Histiocytic sarcoma		1 (100%)				1 (7%)
Cardiovascular System						
Heart	(15)	(15)	(15)	(15)	(15)	(15)
Hemangiosarcoma			1 (7%)			
Endocrine System						
Adrenal medulla	(15)	(15)	(13)	(13)	(15)	(15)
Histiocytic sarcoma						1 (7%)
Pituitary gland	(10)	(13)	(13)	(12)	(11)	(12)
Adenoma		1 (8%)				
General Body System						
None						
Genital System						
Ovary	(15)	(15)	(15)	(15)	(15)	(15)
Histiocytic sarcoma	3 (20%)	2 (13%)	2 (13%)	2 (13%)	2 (13%)	2 (13%)
Uterus	(15)	(15)	(15)	(15)	(15)	(15)
Histiocytic sarcoma		1 (7%)				

TABLE F1
Summary of the Incidence of Neoplasms in Female Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Hematopoietic System						
Bone marrow	(15)					(15)
Histiocytic sarcoma	4 (27%)					3 (20%)
Lymph node, mandibular	(14)	(15)	(13)	(15)	(15)	(15)
Histiocytic sarcoma	1 (7%)					
Lymph node, mesenteric	(14)	(15)	(14)	(15)	(15)	(14)
Histiocytic sarcoma	1 (7%)					
Lymph node, mediastinal	(14)	(15)	(12)	(13)	(12)	(12)
Histiocytic sarcoma	1 (7%)					
Spleen	(15)	(15)	(14)	(15)	(15)	(15)
Hemangiosarcoma			1 (7%)			
Histiocytic sarcoma	1 (7%)					2 (13%)
Thymus	(14)	(15)	(14)	(14)	(15)	(15)
Histiocytic sarcoma	1 (7%)					
Integumentary System						
Mammary gland	(13)	(15)	(15)	(15)	(15)	(15)
Hemangiosarcoma						1 (7%)
Musculoskeletal System						
None						
Nervous System						
Brain	(15)	(14)	(14)	(15)	(15)	(15)
Meninges, histiocytic sarcoma						1 (7%)
Respiratory System						
Lung	(15)	(14)	(14)	(15)	(15)	(15)
Alveolar/bronchiolar adenoma	1 (7%)					
Alveolar/bronchiolar carcinoma						1 (7%)
Histiocytic sarcoma	1 (7%)		1 (7%)			2 (13%)
Special Senses System						
Harderian gland	(15)					(15)
Adenoma						1 (7%)
Urinary System						
None						
Systemic Lesions						
Multiple organs[b]	(15)	(15)	(15)	(15)	(15)	(15)
Histiocytic sarcoma	5 (33%)	2 (13%)	2 (13%)	2 (13%)	2 (13%)	3 (20%)
Leukemia erythrocytic					1 (7%)	

TABLE F1
Summary of the Incidence of Neoplasms in Female Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Neoplasm Summary						
Total animals with primary neoplasms[c]	6	3	5	2	3	6
Total primary neoplasms	6	3	5	2	3	6
Total animals with benign neoplasms	1	1	1			1
Total benign neoplasms	1	1	1			1
Total animals with malignant neoplasms	5	2	4	2	3	5
Total malignant neoplasms	5	2	4	2	3	5

[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 F2
Individual Animal Tumor Pathology of Female Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame: 0 ppm

Number of Days on Study	0 1 3	2 1 9	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 6	2 7 6	2 7 6	2 7 6	2 7 6	2 7 6	2 7 6	2 7 6	
Carcass ID Number	4 8 8	4 9 2	4 8 1	4 8 3	4 8 5	4 8 6	4 8 7	4 8 2	4 8 4	4 8 9	4 9 0	4 9 1	4 9 3	4 9 4	4 9 5	Total Tissues/ Tumors
Alimentary System																
Esophagus	A	+	+	M	+	+	+	+	+	+	+	M	+	+	+	12
Gallbladder	A	A	+	+	+	+	+	M	+	+	+	+	+	+	+	12
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine large, rectum	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Intestine large, cecum	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Intestine small, duodenum	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Intestine small, jejunum	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Intestine small, ileum	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma		X		X												2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Stomach, glandular	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Cardiovascular System																
Blood vessel	M	+	+	+	+	+	+	+	M	+	M	+	+	+	+	12
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Islets, pancreatic	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Parathyroid gland	M	M	M	M	M	+	+	+	+	+	M	M	+	M	+	7
Pituitary gland	M	+	I	+	I	+	I	+	+	M	+	+	+	+	+	10
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
None																
Genital System																
Clitoral gland	M	M	M	M	+	+	M	M	M	+	+	+	+	+	+	8
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma				X		X	X									3
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Vagina	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	14

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

TABLE F2
Individual Animal Tumor Pathology of Female Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame: 0 ppm

Number of Days on Study	0 1 3	2 1 9	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 6	2 7 6	2 7 6	2 7 6	2 7 6	2 7 6	2 7 6	2 7 6	
Carcass ID Number	4 8 8	4 9 2	4 8 1	4 8 3	4 8 5	4 8 6	4 8 7	4 8 2	4 8 4	4 8 9	4 9 0	4 9 1	4 9 3	4 9 4	4 9 5	Total Tissues/ Tumors
Hematopoietic System																
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma				X		X	X				X					4
Lymph node, mandibular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Histiocytic sarcoma		X														1
Lymph node, mesenteric	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Histiocytic sarcoma		X														1
Lymph node, mediastinal	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Histiocytic sarcoma		X														1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma		X														1
Thymus	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Histiocytic sarcoma		X														1
Integumentary System																
Mammary gland	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Skin	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Musculoskeletal System																
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Nervous System																
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Respiratory System																
Larynx	M	+	+	+	M	+	I	M	M	M	I	M	M	M	I	4
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Alveolar/bronchiolar adenoma									X							1
Histiocytic sarcoma		X														1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Special Senses System																
Eye	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Zymbal's gland	+	+	+	M	M	M	+	M	+	+	+	+	+	+	M	10
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Urinary bladder	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma		X		X		X	X				X					5

TABLE F2
Individual Animal Tumor Pathology of Female Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame: 3,125 ppm

Number of Days on Study	275	275	275	275	275	275	275	275	275	275	276	276	276	276	276	
Carcass ID Number	49907	49908	50020	50023	50034	50046	50067	50070	50079	50101	49966	49969	50011	50055	50058	Total Tissues/ Tumors
Alimentary System																
Gallbladder									+							1
Histiocytic sarcoma									X							1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma									X							1
Pancreas									+							1
Histiocytic sarcoma									X							1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pituitary gland	+	+	+	+	+	+	+	+	I	+	+	I	+	+	+	13
Adenoma			X													1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
None																
Genital System																
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma									X						X	2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma									X							1
Hematopoietic System																
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mediastinal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Integumentary System																
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Musculoskeletal System																
None																
Nervous System																
Brain	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	14

TABLE F2
Individual Animal Tumor Pathology of Female Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame: 3,125 ppm

Number of Days on Study	275	275	275	275	275	275	275	275	275	275	275	276	276	276	276	276	
Carcass ID Number	49907	49908	50020	50022	50033	50044	50066	50077	50099	50100	50106	49966	49969	50011	50015	50018	Total Tissues/ Tumors
Respiratory System																	
Lung	+	+	+	+	+	+	+	+	+	+	+		+	+	+		14
Trachea	+											+					2
Special Senses System																	
None																	
Urinary System																	
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Systemic Lesions																	
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma									X							X	2

TABLE F2
Individual Animal Tumor Pathology of Female Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame: 6,250 ppm

Number of Days on Study	1 0 8	2 1 6	2 7 5	2 7 5	2 7 5	2 7 5	2 7 6	2 7 6	2 7 6	2 7 6	2 7 6	2 7 6	2 7 6	2 7 6	2 7 6	
Carcass ID Number	5 1 7	5 1 6	5 1 1	5 2 0	5 2 3	5 2 5	5 1 2	5 1 3	5 1 4	5 1 5	5 1 8	5 1 9	5 2 1	5 2 2	5 2 4	Total Tissues/ Tumors
Alimentary System																
Intestine large, cecum	A												+			1
Leiomyoma													X			1
Intestine small, jejunum	A															
Intestine small, ileum	A															
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma				X												1
Stomach, forestomach	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Stomach, glandular	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hemangiosarcoma											X					1
Endocrine System																
Adrenal cortex	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Adrenal medulla	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Pituitary gland	I	I	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
Tissue NOS		+														1
Genital System																
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma				X		X										2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Lymph node, mandibular	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Lymph node, mesenteric	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mediastinal	A	A	+	+	+	+	+	+	+	+	+	+	I	+	+	12
Spleen	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Hemangiosarcoma	X															1
Thymus	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Integumentary System																
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Musculoskeletal System																
None																
Nervous System																
Brain	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14

TABLE F2
Individual Animal Tumor Pathology of Female Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame: 6,250 ppm

Number of Days on Study	1 0 8	2 1 6	2 7 5	2 7 5	2 7 5	2 7 5	2 7 6	2 7 6	2 7 6	2 7 6	2 7 6	2 7 6	2 7 6	2 7 6	2 7 6	
Carcass ID Number	5 1 7	5 1 6	5 1 1	5 2 0	5 2 3	5 2 5	5 1 2	5 1 3	5 1 4	5 1 5	5 1 8	5 1 9	5 2 1	5 2 2	5 2 4	Total Tissues/ Tumors
Respiratory System																
Lung	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Histiocytic sarcoma				X												1
Special Senses System																
None																
Urinary System																
Kidney	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Urinary bladder		+														1
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma				X		X										2

TABLE F2
Individual Animal Tumor Pathology of Female Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame: 12,500 ppm

Number of Days on Study	27575	27756	27756	27766	27766	27766	27766	27766	27766	27766	27766	27766	27766	27766	27766	
Carcass ID Number	52828	52929	53033	52636	52737	53040	53341	53342	53344	53345	53346	53347	53348	53349	53440	Total Tissues/ Tumors
Alimentary System																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	13
Pituitary gland	I	+	+	+	+	+	+	+	+	+	M	+	+	+	I	12
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
None																
Genital System																
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma							X				X					2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mediastinal	+	+	+	+	+	I	+	+	+	+	+	+	+	M	+	13
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	14
Integumentary System																
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Musculoskeletal System																
None																
Nervous System																
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Respiratory System																
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE F2
Individual Animal Tumor Pathology of Female Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame: 12,500 ppm

Number of Days on Study	275	275	275	276	276	276	276	276	276	276	276	276	276	276	276	276
Carcass ID Number	52 8	52 9	53 3	52 6	52 7	53 0	53 1	53 2	53 4	53 5	53 6	53 7	53 8	53 9	54 0	Total Tissues/ Tumors
Special Senses System																
None																
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma							X				X					2

TABLE F2
Individual Animal Tumor Pathology of Female Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame: 25,000 ppm

Number of Days on Study	27575	27575	27575	27575	27575	27575	27576	27576	27576	27576	27576	27576	27576	27576	27576	
Carcass ID Number	54422	54435	54455	55511	55522	55525	54411	54444	54446	54447	54448	54449	55500	55503	55504	Total Tissues/ Tumors
Alimentary System																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pituitary gland	+	I	+	+	+	+	+	I	I	+	+	M	+	+	+	11
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
None																
Genital System																
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma									X		X					2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mediastinal	M	I	+	+	+	+	+	+	+	+	+	+	+	+	M	12
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Integumentary System																
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Musculoskeletal System																
None																
Nervous System																
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Respiratory System																
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE F2
Individual Animal Tumor Pathology of Female Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame: 25,000 ppm

Number of Days on Study	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 5	2 7 6	2 7 6	2 7 6	2 7 6	2 7 6	2 7 6	2 7 6	2 7 6	2 7 6	2 7 6	
Carcass ID Number	5 4 2	5 4 3	5 4 5	5 5 1	5 5 2	5 5 5	5 4 1	5 4 4	5 4 6	5 4 7	5 4 8	5 4 9	5 5 0	5 5 3	5 5 4	Total Tissues/ Tumors	
Special Senses System																	
None																	
Urinary System																	
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15	
Systemic Lesions																	
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15	
Histiocytic sarcoma									X		X					2	
Leukemia erythrocytic					X											1	

TABLE F2
Individual Animal Tumor Pathology of Female Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame: 50,000 ppm

Number of Days on Study	267	275	275	275	275	275	275	276	276	276	276	276	276	276	276	
Carcass ID Number	56 0	55 8	55 9	56 1	56 4	56 8	56 9	55 6	55 7	56 2	56 3	56 5	56 6	56 7	57 0	Total Tissues/ Tumors
Alimentary System																
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Gallbladder	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	14
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma	X												X			2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma													X			1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Cardiovascular System																
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma													X			1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Parathyroid gland	+	+	+	M	M	M	+	M	M	M	M	M	M	M	M	4
Pituitary gland	+	+	+	+	+	M	+	+	+	I	M	+	+	+	+	12
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
None																
Genital System																
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma	X												X			2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE F2
Individual Animal Tumor Pathology of Female Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame: 50,000 ppm

Number of Days on Study	267	275	275	275	275	275	275	276	276	276	276	276	276	276	276	
Carcass ID Number	56 0	55 8	55 9	56 1	56 4	56 8	56 9	56 5	55 7	56 2	66 3	66 5	66 6	66 7	57 0	Total Tissues/ Tumors
Hematopoietic System																
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma	X		X										X			3
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	14
Lymph node, mediastinal	+	+	+	M	+	+	+	+	+	+	+	M	+	M	+	12
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma	X												X			2
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Integumentary System																
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hemangiosarcoma				X												1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Musculoskeletal System																
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Nervous System																
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Meninges, histiocytic sarcoma	X															1
Peripheral nerve	+															1
Spinal cord	+															1
Respiratory System																
Larynx	I	+	I	I	+	I	+	I	M	I	I	M	+	I	+	5
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Alveolar/bronchiolar carcinoma														X		1
Histiocytic sarcoma	X												X			2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Special Senses System																
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adenoma					X											1
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Histiocytic sarcoma	X		X										X			3

TABLE F3
Statistical Analysis of Primary Neoplasms in Female Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
All Organs: Hemangiosarcoma						
Overall rate[a]	0/15 (0%)	0/15 (0%)	2/15 (13%)	0/15 (0%)	0/15 (0%)	1/15 (7%)
Adjusted rate[b]	0.0%	0.0%	13.8%	0.0%	0.0%	6.7%
Terminal rate[c]	0/13 (0%)	0/15 (0%)	1/13 (8%)	0/15 (0%)	0/15 (0%)	1/14 (7%)
First incidence (days)	—[e]	—[f]	108	—	—	275 (T)
Poly-3 test[d]	P=0.494	—	P=0.247	—	—	P=0.520
All Organs: Histiocytic Sarcoma						
Overall rate	5/15 (33%)	2/15 (13%)	2/15 (13%)	2/15 (13%)	2/15 (13%)	3/15 (20%)
Adjusted rate	35.7%	13.3%	14.8%	13.3%	13.3%	20.0%
Terminal rate	4/13 (31%)	2/15 (13%)	2/13 (15%)	2/15 (13%)	2/15 (13%)	2/14 (14%)
First incidence (days)	219	275 (T)	275 (T)	275 (T)	275 (T)	267
Poly-3 test	P=0.465N	P=0.166N	P=0.206N	P=0.166N	P=0.166N	P=0.302N
All Organs: Malignant Neoplasms						
Overall rate	5/15 (33%)	2/15 (13%)	4/15 (27%)	2/15 (13%)	3/15 (20%)	5/15 (33%)
Adjusted rate	35.7%	13.3%	27.6%	13.3%	20.0%	33.3%
Terminal rate	4/13 (31%)	2/15 (13%)	3/13 (23%)	2/15 (13%)	3/15 (20%)	4/14 (29%)
First incidence (days)	219	275 (T)	108	275 (T)	275 (T)	267
Poly-3 test	P=0.349	P=0.166N	P=0.476N	P=0.166N	P=0.302N	P=0.598N
All Organs: Benign or Malignant Neoplasms						
Overall rate	6/15 (40%)	3/15 (20%)	5/15 (33%)	2/15 (13%)	3/15 (20%)	6/15 (40%)
Adjusted rate	42.9%	20.0%	34.5%	13.3%	20.0%	40.0%
Terminal rate	5/13 (39%)	3/15 (20%)	4/13 (31%)	2/15 (13%)	3/15 (20%)	5/14 (36%)
First incidence (days)	219	275 (T)	108	275 (T)	275 (T)	267
Poly-3 test	P=0.385	P=0.178N	P=0.472N	P=0.082N	P=0.178N	P=0.585N

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

TABLE F4
Summary of the Incidence of Noneoplastic Lesions in Female Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame[a]

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Disposition Summary						
Animals initially in study	15	15	15	15	15	15
Early deaths						
Moribund sacrifice						1
Natural death	2		2			
Survivors						
Terminal sacrifice	13	15	13	15	15	14
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Liver	(15)	(15)	(15)	(15)	(15)	(15)
Hematopoietic cell proliferation						2 (13%)
Infiltration cellular, focal, lymphocyte						1 (7%)
Infiltration cellular, lymphocyte	1 (7%)					
Inflammation, chronic active	1 (7%)					
Necrosis, focal					1 (7%)	2 (13%)
Hepatocyte, necrosis, focal		1 (7%)				
Hepatocyte, periportal, vacuolization cytoplasmic						2 (13%)
Salivary glands	(15					(15)
Infiltration cellular, focal, lymphocyte	4 (27%)					5 (33%)
Cardiovascular System						
Heart	(15)	(15)	(15)	(15)	(15)	(15)
Myocardium, necrosis, focal			1 (7%)			
Endocrine System						
Adrenal cortex	(15)	(15)	(13)	(15)	(15)	(15)
Accessory adrenal cortical nodule					1 (7%)	
Subcapsular, hyperplasia, focal	14 (93%)	15 (100%)	12 (92%)	15 (100%)	15 (100%)	15 (100%)
Thyroid gland	(15)	(15)	(15)	(15)	(15)	(15)
Ectopic thymus				2 (13%)		
Ultimobranchial cyst	1 (7%)					
General Body System						
Tissue NOS			(1)			
Abdominal, fat, hemorrhage, focal			1 (100%)			
Genital System						
Ovary	(15)	(15)	(15)	(15)	(15)	(15)
Cyst				1 (7%)		
Uterus	(15)	(15)	(15)	(15)	(15)	(15)
Endometrium, hyperplasia, cystic	14 (93%)	13 (87%)	13 (87%)	15 (100%)	15 (100%)	14 (93%)

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

TABLE F4
Summary of the Incidence of Noneoplastic Lesions in Female Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Hematopoietic System						
Lymph node, mandibular	(14)	(15)	(13)	(15)	(15)	(15)
Hyperplasia						1 (7%)
Inflammation, chronic active	1 (7%)					
Lymph node, mesenteric	(14)	(15)	(14)	(15)	(15)	(14)
Hyperplasia, histiocytic						1 (7%)
Spleen	(15)	(15)	(14)	(15)	(15)	(15)
Accessory spleen				1 (7%)		
Hematopoietic cell proliferation	5 (33%)	2 (13%)	4 (29%)	3 (20%)	2 (13%)	3 (20%)
Pigmentation	1 (7%)					
Thymus	(14)	(15)	(14)	(14)	(15)	(15)
Atrophy, diffuse	1 (7%)					
Atrophy, focal		1 (7%)	1 (7%)			
Integumentary System						
None						
Musculoskeletal System						
Bone	(15)					(15)
Cranium, osteoporosis	1 (7%)					
Nervous System						
Brain	(15)	(14)	(14)	(15)	(15)	(15)
Granuloma	1 (7%)					
Cerebellum, medulla, vacuolization cytoplasmic	1 (7%)					
Spinal cord						(1)
Vacuolization cytoplasmic						1 (100%)
Respiratory System						
Lung	(15)	(14)	(14)	(15)	(15)	(15)
Alveolar epithelium, hyperplasia, focal	1 (7%)					
Alveolus, crystals	1 (7%)					
Nose	(15)					(15)
Olfactory epithelium, atrophy						1 (7%)
Olfactory epithelium, hyaline droplet	2 (13%)					3 (20%)
Respiratory epithelium, hyaline droplet	1 (7%)					2 (13%)
Special Senses System						
Eye	(14)					(15)
Cataract	1 (7%)					
Harderian gland	(15)					(15)
Hyperplasia						1 (7%)
Infiltration cellular, lymphocyte						1 (7%)

TABLE F4
Summary of the Incidence of Noneoplastic Lesions in Female Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Urinary System						
Kidney	(15)	(15)	(13)	(15)	(15)	(15)
Nephropathy	1 (7%)					1 (7%)
Glomerulus, hyalinization						1 (7%)
Renal tubule, dilatation, diffuse	1 (7%)	1 (7%)			1 (7%)	
Renal tubule, dilatation, focal	10 (67%)	7 (47%)	5 (38%)	5 (33%)	5 (33%)	9 (60%)

APPENDIX G
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Zeiger *et al.* (1992). Aspartame was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA97, TA98, TA100, TA1535, and TA1537 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of aspartame. In the absence of toxicity, 10,000 µg/plate was selected as the high dose. All trials were repeated at the same or a higher S9 fraction.

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

RAT BONE MARROW MICRONUCLEUS TEST PROTOCOL

Preliminary range-finding studies were performed. Factors affecting dose selection included chemical solubility and toxicity and the extent of cell cycle delay induced by aspartame exposure. The standard three-exposure protocol is described in detail by Shelby *et al.* (1993). Male F344/N rats were given aspartame dissolved in corn oil by gavage [three times at 24-hour intervals]. Vehicle control animals were gavaged with corn oil only. The positive control animals received cyclophosphamide. The animals were killed 24 hours after the third dose, and blood smears were prepared from bone marrow cells obtained from the femurs. Air-dried smears were fixed and stained; 2,000 polychromatic erythrocytes (PCEs) were scored for the frequency of micronucleated cells in each of five animals per dose group. In addition, the percentage of PCEs among 200 erythrocytes in the bone marrow of each animal was scored as a measure of toxicity.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among PCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dose group is less than or equal to 0.025 divided by the number of dose groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 9-month toxicity studies, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs) in up to 12 Tg.AC hemizygous, 15 p53 haploinsufficient, and 15 Cdkn2a deficient mice per exposure group. In addition, the percent of PCEs among 1000 erythrocytes in peripheral blood was scored as a measure of toxicity.

The results were tabulated as described for PCEs in the rat bone marrow micronucleus test. Results of the 9-month studies were accepted without repeat tests, because additional test data could not be obtained.

Evaluation Protocol

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

Results

Aspartame (100 to 10,000 µg/plate) was tested for induction of gene mutations in *S. typhimurium* with and without induced rat or hamster liver S9 metabolic activation enzymes (Table G1). No mutagenicity was detected in strains TA98, TA100, or TA1535 with or without S9. In addition, a single test in TA1537 with 30% rat liver S9 also gave negative results. In TA97 with 30% rat liver S9, however, a reproducible small increase in mutant colonies was observed, and this response was judged to be equivocal. No mutagenicity was detected in TA97 without S9 or with hamster liver S9.

No increase in micronucleated PCEs was observed in bone marrow of male F344/N rats administered aspartame by gavage over a dose range of 500 to 2,000 mg/kg (Table G2). The percent of PCEs was not greatly altered in treated rats (Table G2). However, a slight decrease in percent PCEs occurred in the 1,000 mg/kg group, and in the positive controls the percent of PCEs was dramatically lowered, an indication of marked toxicity.

Peripheral blood micronucleus tests were conducted in Tg.AC hemizygous, p53 haploinsufficient, and Cdkn2a deficient mice after 9 months exposure to 3,125 to 50,000 ppm aspartame in feed. Negative results were obtained in male and female Tg.AC hemizygous and Cdkn2a deficient mice (Tables G3 and G4). Negative results were also obtained in male p53 haploinsufficient mice (Table G5); in female p53 haploinsufficient mice, the results of the micronucleus test were judged to be positive based on a significant trend test and the increased frequency of micronucleated erythrocytes seen in the 50,000 ppm group. For all three strains of mice, the percent PCEs was not significantly altered by treatment with aspartame.

TABLE G1
Mutagenicity of Aspartame in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	+hamster S9 10%	+hamster S9 30%
TA100	0	143 ± 7.2	105 ± 3.7	108 ± 9.4	152 ± 12.0
	100	159 ± 5.0	129 ± 7.6	141 ± 14.5	168 ± 4.4
	333	181 ± 8.1	127 ± 11.2	118 ± 5.8	163 ± 6.7
	1,000	185 ± 11.1	111 ± 6.7	113 ± 1.2	148 ± 5.9
	3,333	170 ± 14.7	113 ± 6.6	129 ± 7.2	159 ± 19.3
	10,000	178 ± 6.1	122 ± 8.4	119 ± 10.7	191 ± 16.5
Trial summary		Negative	Negative	Negative	Negative
Positive control		312 ± 24.8	219 ± 15.0	812 ± 41.6	458 ± 15.3

		+rat S9 10%	+rat S9 30%	+rat S9 30%	+rat S9 30%
TA100	0	115 ± 8.5	97 ± 5.2	95 ± 7.5	80 ± 5.0
(continued)	100	118 ± 2.6	96 ± 4.6		
	333	114 ± 7.9	103 ± 6.4	89 ± 6.8	110 ± 11.7
	1,000	116 ± 10.7	112 ± 4.7	96 ± 9.0	101 ± 11.4
	3,333	127 ± 8.6	109 ± 6.4	96 ± 3.0	81 ± 5.7
	6,667			80 ± 3.8	80 ± 6.7
	10,000	127 ± 6.6	202 ± 39.4	77 ± 5.5	81 ± 3.5
Trial summary		Negative	Equivocal	Negative	Negative
Positive control		586 ± 36.	633 ± 30.3	636 ± 47.3	180 ± 9.4

		–S9 Trial 1	–S9 Trial 2	+hamster S9 10%	+hamster S9 30%
TA1535	0	13 ± 1.5	18 ± 2.9	13 ± 0.6	16 ± 2.0
	100	10 ± 2.0	12 ± 3.5	11 ± 0.7	10 ± 1.5
	333	19 ± 2.3	11 ± 2.6	12 ± 1.9	10 ± 1.0
	1,000	11 ± 0.9	12 ± 2.4	13 ± 1.5	10 ± 1.2
	3,333	11 ± 0.9	14 ± 4.0	10 ± 2.7	10 ± 2.9
	10,000	13 ± 1.5	14 ± 2.1	11 ± 5.6	12 ± 2.1
Trial summary		Negative	Negative	Negative	Negative
Positive control		108 ± 4.8	166 ± 3.2	91 ± 5.6	97 ± 4.7

		+rat S9 10%	+rat S9 30%	+rat S9 30%
TA1535	0	13 ± 2.8	9 ± 1.8	12 ± 1.2
(continued)	100	15 ± 0.6	20 ± 3.4	12 ± 3.0
	333	11 ± 1.8	17 ± 4.8	15 ± 5.0
	1,000	12 ± 0.3	20 ± 3.4	12 ± 1.7
	3,333	7 ± 2.0	21 ± 2.6	14 ± 1.5
	10,000	10 ± 0.3	22 ± 1.7	13 ± 0.9
Trial summary		Negative	Equivocal	Negative
Positive control		172 ± 1.2	73 ± 6.6	269 ± 3.41

TABLE G1
Mutagenicity of Aspartame in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate +rat S9 30%
TA1537	0	15 ± 0.3
	100	10 ± 3.5
	333	9 ± 0.7
	1,000	8 ± 1.2
	3,333	15 ± 1.5
	10,000	8 ± 0.9
Trial summary		Negative
Positive control		57 ± 13.1

Strain	Dose (µg/plate)	–S9 Trial 1	–S9 Trial 2	+hamster S9 10%	+hamster S9 30%
TA97	0	105 ± 7.3	140 ± 12.1	141 ± 9.2	140 ± 2.9
	100	122 ± 5.0	122 ± 0.3	135 ± 6.7	142 ± 1.9
	333	108 ± 7.2	125 ± 10.2	141 ± 5.6	124 ± 8.7
	1,000	122 ± 12.3	124 ± 2.6	135 ± 3.9	142 ± 10.8
	3,333	103 ± 7.3	147 ± 5.4	118 ± 8.1	140 ± 4.1
	10,000	107 ± 3.2	142 ± 6.0	132 ± 14.5	142 ± 10.1
Trial summary		Negative	Negative	Negative	Negative
Positive control		354 ± 11.5	292 ± 13.0	1,062 ± 35.1	539 ± 73.6

Strain	Dose (µg/plate)	+rat S9 10%	+rat S9 30%	+rat S9 30%
TA97 (continued)	0	153 ± 7.3	154 ± 22.9	126 ± 5.5
	100	138 ± 9.6	150 ± 13.9	131 ± 14.1
	333	125 ± 8.1	176 ± 10.7	128 ± 19.0
	1,000	152 ± 5.0	179 ± 10.7	173 ± 29.0
	3,333	130 ± 6.9	193 ± 8.1	168 ± 1.5
	10,000	149 ± 7.6	204 ± 15.1	170 ± 19.2
Trial summary		Negative	Equivocal	Equivocal
Positive control		898 ± 121.3	363 ± 25.4	732 ± 94.4

Strain	Dose (µg/plate)	–S9 Trial 1	–S9 Trial 2	+hamster S9 10%	+hamster S9 30%	+rat S9 10%	+rat S9 30%
TA98	0	11 ± 0.7	28 ± 2.8	36 ± 5.2	20 ± 1.8	37 ± 0.9	23 ± 1.8
	100	12 ± 1.7	30 ± 4.7	34 ± 1.8	24 ± 0.9	29 ± 6.6	22 ± 2.0
	333	17 ± 1.0	23 ± 2.8	27 ± 6.5	23 ± 2.7	31 ± 3.9	16 ± 2.8
	1,000	14 ± 0.9	23 ± 2.7	31 ± 5.1	24 ± 3.2	35 ± 4.9	12 ± 1.7
	3,333	17 ± 1.3	25 ± 2.6	32 ± 5.8	24 ± 2.2	33 ± 3.6	16 ± 1.2
	10,000	12 ± 0.9	30 ± 0.7	32 ± 1.5	20 ± 1.7	35 ± 1.5	15 ± 0.6
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		211 ± 3.8	205 ± 11.0	878 ± 36.0	523 ± 26.4	422 ± 14.2	147 ± 14.2

[a] Study performed at BioReliance. The detailed protocol is presented by Zeiger *et al*. (1992). 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracine.

TABLE G2
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Rats Treated with Aspartame by Gavage[a]

Compound	Dose (mg/kg)	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	P Value[c]	PCEs (%)
Corn oil[d]		5	1.40 ± 0.41		60.8
Aspartame	500	5	1.10 ± 0.33	0.7259	57.5
	1,000	5	1.10 ± 0.22	0.7259	39.0
	2,000	5	0.80 ± 0.41	0.8997	55.4
			P=0.889[e]		
Cyclophosphamide[f]	25	5	34.50 ± 8.31		10.7

[a] Study was performed at ILS, Inc. The detailed protocol is presented by Shelby *et al*. (1993); PCE=polychromatic erythrocyte.
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control, significant at P≤0.008 (ILS, 1990).
[d] Vehicle control
[e] Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)
[f] Positive control

TABLE G3
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Tg.AC Hemizygous Mice Following Administration of Aspartame in Feed for 9 Months[a]

Dose (ppm)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	% PCE
Male				
NTP-2000 feed[d]	9	0.67 ± 0.25		2.28
3,125	11	0.82 ± 0.18	0.2909	1.97
6,250	8	1.38 ± 0.31	0.0196	2.01
12,500	12	0.88 ± 0.29	0.2254	1.97
25,000	11	0.86 ± 0.12	0.2406	2.08
50,000	10	0.95 ± 0.17	0.1670	2.10
		P=0.401[e]		
Female				
NTP-2000 feed	11	0.77 ± 0.21		2.50
3,125	10	0.60 ± 0.15	0.7495	2.44
6,250	8	0.75 ± 0.16	0.5316	2.33
12,500	9	0.89 ± 0.22	0.3436	2.63
25,000	11	0.77 ± 0.18	0.5000	2.61
50,000	9	1.22 ± 0.29	0.0759	2.74
		P=0.028		

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

TABLE G4
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Cdkn2a Deficient Mice Following Administration of Aspartame in Feed for 9 Months[a]

Dose (ppm)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	% PCE
Male				
NTP-2000 feed[d]	14	1.86 ± 0.19		2.74
3,125	14	1.82 ± 0.24	0.5393	2.28
6,250	15	2.00 ± 0.20	0.3477	2.62
12,500	14	1.54 ± 0.19	0.8223	2.54
25,000	14	1.82 ± 0.25	0.5393	2.48
50,000	15	1.50 ± 0.21	0.8536	2.60
		P=0.886[e]		
Female				
NTP-2000 feed	13	1.19 ± 0.20		2.75
3,125	15	1.17 ± 0.20	0.5351	2.79
6,250	13	1.42 ± 0.26	0.2333	3.14
12,500	15	1.40 ± 0.27	0.2485	2.68
25,000	15	1.40 ± 0.15	0.2485	3.17
50,000	14	1.14 ± 0.19	0.5668	3.29
		P=0.605		

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

TABLE G5
Frequency of Micronuclei in Peripheral Blood Erythrocytes of p53 Haploinsufficient Mice Following Administration of Aspartame in Feed for 9 Months[a]

Dose (ppm)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	% PCE
Male				
NTP-2000 feed[d]	14	1.18 ± 0.19		2.23
3,125	15	1.40 ± 0.20	0.2292	2.43
6,250	13	1.27 ± 0.25	0.3816	2.77
12,500	15	1.27 ± 0.18	0.3809	2.50
25,000	14	1.57 ± 0.16	0.1048	2.49
50,000	14	1.43 ± 0.22	0.2062	2.82
		P=0.201[e]		
Female				
NTP-2000 feed	14	0.79 ± 0.14		3.33
3,125	14	1.04 ± 0.20	0.1634	2.93
6,250	14	0.96 ± 0.15	0.2374	2.81
12,500	15	1.13 ± 0.17	0.0890	2.85
25,000	15	1.03 ± 0.17	0.1620	2.83
50,000	15	1.80 ± 0.20	0.0004	2.99
		P=0.000		

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

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

TABLE H1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame[a]

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Male						
n	9	12	8	12	11	10
Necropsy body wt	32.8 ± 0.4	31.3 ± 0.7	32.1 ± 0.8	31.2 ± 0.7	31.2 ± 1.1	31.7 ± 0.6
Brain						
Absolute	0.492 ± 0.007	0.507 ± 0.007	0.521 ± 0.012	0.510 ± 0.007	0.514 ± 0.010	0.525 ± 0.007*
Relative	15.025 ± 0.221	16.297 ± 0.428	16.302 ± 0.483	16.436 ± 0.322	16.634 ± 0.540*	16.608 ± 0.379
Heart						
Absolute	0.154 ± 0.002	0.157 ± 0.003	0.164 ± 0.004	0.155 ± 0.003	0.163 ± 0.006	0.157 ± 0.002
Relative	4.692 ± 0.064	5.022 ± 0.033*	5.115 ± 0.104*	5.000 ± 0.094	5.256 ± 0.120**	4.959 ± 0.088
R. Kidney						
Absolute	0.265 ± 0.006	0.262 ± 0.008	0.264 ± 0.012	0.260 ± 0.008[b]	0.279 ± 0.006	0.290 ± 0.012
Relative	8.092 ± 0.115	8.398 ± 0.226	8.273 ± 0.401	8.382 ± 0.281[b]	9.013 ± 0.237*	9.136 ± 0.287*
Liver						
Absolute	1.549 ± 0.026	1.373 ± 0.051	1.405 ± 0.040	1.375 ± 0.034	1.425 ± 0.069	1.502 ± 0.063
Relative	47.3 ± 1.1	43.8 ± 0.9	43.9 ± 1.1	44.1 ± 0.6	45.6 ± 1.4	47.4 ± 1.8
Lung						
Absolute	0.241 ± 0.015	0.217 ± 0.017	0.189 ± 0.007*	0.202 ± 0.008	0.211 ± 0.010	0.206 ± 0.006
Relative	7.331 ± 0.428	6.956 ± 0.493	5.905 ± 0.195	6.512 ± 0.268	6.814 ± 0.304	6.530 ± 0.72
R. Testis						
Absolute	0.090 ± 0.001	0.077 ± 0.006	0.084 ± 0.003	0.087 ± 0.002	0.088 ± 0.002	0.082 ± 0.004
Relative	2.748 ± 0.051	2.466 ± 0.196	2.624 ± 0.122	2.807 ± 0.078	2.854 ± 0.133	2.582 ± 0.141
Thymus						
Absolute	0.027 ± 0.003	0.021 ± 0.002	0.022 ± 0.001	0.022 ± 0.002	0.024 ± 0.003	0.021 ± 0.002
Relative	0.825 ± 0.089	0.666 ± 0.054	0.671 ± 0.039	0.694 ± 0.066	0.760 ± 0.071	0.670 ± 0.058

TABLE H1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Female						
n	11	10	9	9	11	8
Necropsy body wt	26.5 ± 0.5	26.1 ± 0.4	27.2 ± 0.8	26.4 ± 0.9	26.7 ± 0.7	27.8 ± 1.4
Brain						
Absolute	0.509 ± 0.011	0.488 ± 0.005	0.509 ± 0.008	0.516 ± 0.003	0.506 ± 0.006	0.520 ± 0.011
Relative	19.257 ± 0.481	18.756 ± 0.320	18.754 ± 0.352	19.744 ± 0.615	19.092 ± 0.477	19.206 ± 1.492
Heart						
Absolute	0.125 ± 0.003	0.121 ± 0.002	0.127 ± 0.004	0.123 ± 0.003	0.120 ± 0.003	0.125 ± 0.005
Relative	4.726 ± 0.115	4.628 ± 0.095	4.682 ± 0.102	4.702 ± 0.137	4.529 ± 0.154	4.594 ± 0.326
R. Kidney						
Absolute	0.188 ± 0.005	0.183 ± 0.005	0.191 ± 0.007	0.199 ± 0.011	0.186 ± 0.005	0.189 ± 0.003
Relative	7.089 ± 0.178	7.001 ± 0.162	7.009 ± 0.192	7.562 ± 0.340	7.016 ± 0.251	6.932 ± 0.427
Liver						
Absolute	1.279 ± 0.045	1.208 ± 0.035	1.259 ± 0.061	1.201 ± 0.045[c]	1.207 ± 0.033	1.351 ± 0.042
Relative	48.2 ± 1.3	46.3 ± 1.0	46.1 ± 1.2	46.9 ± 1.7[c]	45.4 ± 1.3	49.4 ± 2.7
Lung						
Absolute	0.192 ± 0.007	0.225 ± 0.018	0.196 ± 0.012	0.207 ± 0.026	0.203 ± 0.008	0.209 ± 0.009
Relative	7.269 ± 0.312	8.636 ± 0.713	7.298 ± 0.591	7.934 ± 1.017	7.685 ± 0.428	7.624 ± 0.459
Thymus						
Absolute	0.026 ± 0.001	0.024 ± 0.002	0.026 ± 0.003	0.028 ± 0.002	0.025 ± 0.002	0.031 ± 0.002
Relative	0.983 ± 0.054	0.937 ± 0.080	0.965 ± 0.096	1.058 ± 0.056	0.930 ± 0.080	1.134 ± 0.082

* Significantly different ($P \leq 0.05$) from the chamber control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n = 11
[c] n = 8

TABLE H2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame[a]

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Male						
n	14	15	13	15	14	14
Necropsy body wt	31.3 ± 1.2	30.4 ± 0.7	29.8 ± 0.8	30.0 ± 0.9	30.1 ± 0.8	30.1 ± 0.8
Brain						
Absolute	0.470 ± 0.008	0.474 ± 0.005	0.465 ± 0.006	0.464 ± 0.007	0.482 ± 0.006	0.464 ± 0.006
Relative	15.244 ± 0.489	15.733 ± 0.379	15.715 ± 0.410	15.610 ± 0.418	16.144 ± 0.460	15.530 ± 0.401
Heart						
Absolute	0.161 ± 0.004	0.162 ± 0.002	0.160 ± 0.004	0.158 ± 0.004	0.165 ± 0.003	0.157 ± 0.002
Relative	5.208 ± 0.143	5.372 ± 0.147	5.383 ± 0.110	5.306 ± 0.127	5.510 ± 0.140	5.234 ± 0.130
R. Kidney						
Absolute	0.237 ± 0.008	0.260 ± 0.005	0.245 ± 0.007	0.245 ± 0.008	0.250 ± 0.006	0.245 ± 0.007
Relative	7.677 ± 0.300	8.615 ± 0.190*	8.242 ± 0.163	8.190 ± 0.209	8.350 ± 0.236	8.157 ± 0.228
Liver						
Absolute	1.359 ± 0.033	1.361 ± 0.036	1.334 ± 0.035	1.369 ± 0.050	1.378 ± 0.045	1.400 ± 0.059
Relative	43.8 ± 0.8	44.9 ± 0.7	44.9 ± 0.8	45.6 ± 0.9	45.8 ± 0.9	46.4 ± 1.4
Lung						
Absolute	0.211 ± 0.009	0.223 ± 0.007	0.212 ± 0.014	0.225 ± 0.012	0.208 ± 0.007	0.206 ± 0.008
Relative	6.803 ± 0.272	7.366 ± 0.154	7.128 ± 0.406	7.520 ± 0.380	6.953 ± 0.267	6.854 ± 0.232
R. Testis						
Absolute	0.109 ± 0.002	0.110 ± 0.003	0.109 ± 0.002	0.104 ± 0.004	0.108 ± 0.002	0.105 ± 0.002
Relative	3.527 ± 0.117	3.646 ± 0.080	3.666 ± 0.076	3.499 ± 0.145	3.600 ± 0.100	3.520 ± 0.124
Thymus						
Absolute	0.041 ± 0.003	0.038 ± 0.001	0.038 ± 0.003	0.040 ± 0.002	0.036 ± 0.002	0.036 ± 0.002
Relative	1.297 ± 0.085	1.263 ± 0.037	1.291 ± 0.095	1.327 ± 0.056	1.210 ± 0.071	1.186 ± 0.070

TABLE H2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Female						
n	14	14	14	15	15	15
Necropsy body wt	26.1 ± 0.8	24.6 ± 0.5	26.4 ± 1.0	25.8 ± 0.8	24.8 ± 0.4	25.8 ± 0.7
Brain						
Absolute	0.481 ± 0.004	0.475 ± 0.007	0.483 ± 0.006	0.483 ± 0.004	0.478 ± 0.005	0.476 ± 0.005
Relative	18.660 ± 0.550	19.419 ± 0.398	18.521 ± 0.529	18.990 ± 0.610	19.329 ± 0.336	18.644 ± 0.466
Heart						
Absolute	0.142 ± 0.006	0.140 ± 0.006	0.140 ± 0.004	0.135 ± 0.003	0.138 ± 0.003	0.137 ± 0.003
Relative	5.437 ± 0.118	5.684 ± 0.150	5.355 ± 0.128	5.290 ± 0.140	5.564 ± 0.102	5.338 ± 0.132
R. Kidney						
Absolute	0.183 ± 0.004	0.182 ± 0.006	0.195 ± 0.005	0.187 ± 0.005	0.195 ± 0.003	0.187 ± 0.004
Relative	7.069 ± 0.161	7.416 ± 0.189	7.434 ± 0.199	7.291 ± 0.164	7.872 ± 0.138*	7.340 ± 0.252
Liver						
Absolute	1.221 ± 0.026[b]	1.181 ± 0.038	1.209 ± 0.043	1.184 ± 0.042	1.162 ± 0.023	1.185 ± 0.039
Relative	48.2 ± 1.1[b]	48.1 ± 1.0	46.0 ± 1.1	46.0 ± 1.0	46.9 ± 0.9	46.0 ± 0.9
Lung						
Absolute	0.199 ± 0.006	0.200 ± 0.004	0.197 ± 0.008	0.207 ± 0.005	0.189 ± 0.004	0.203 ± 0.006
Relative	7.725 ± 0.339	8.204 ± 0.225	7.502 ± 0.272	8.101 ± 0.261	7.639 ± 0.214	7.958 ± 0.300
Thymus						
Absolute	0.045 ± 0.004[b]	0.042 ± 0.002	0.048 ± 0.003	0.042 ± 0.002	0.043 ± 0.003	0.046 ± 0.002
Relative	1.760 ± 0.161[b]	1.707 ± 0.101	1.820 ± 0.077	1.658 ± 0.081	1.747 ± 0.101	1.774 ± 0.091

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

[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

[b] n = 13

TABLE H3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame[a]

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Male						
n	14	14	15	14	14	15
Necropsy body wt	35.86 ± 1.08	33.56 ± 0.72	34.29 ± 0.96	35.36 ± 1.21	35.69 ± 0.87	34.84 ± 1.13
Brain						
Absolute	0.478 ± 0.005	0.478 ± 0.005	0.486 ± 0.006	0.483 ± 0.004	0.489 ± 0.006	0.474 ± 0.007
Relative	13.482 ± 0.442	14.331 ± 0.384	14.331 ± 0.463	13.896 ± 0.574	13.823 ± 0.415	13.764 ± 0.393
Heart						
Absolute	0.193 ± 0.005	0.181 ± 0.004	0.178 ± 0.005	0.182 ± 0.006	0.189 ± 0.006	0.192 ± 0.005
Relative	5.452 ± 0.235	5.403 ± 0.122	5.215 ± 0.111	5.194 ± 0.143	5.323 ± 0.185	5.565 ± 0.200
R. Kidney						
Absolute	0.316 ± 0.009	0.312 ± 0.009	0.305 ± 0.007	0.315 ± 0.008	0.318 ± 0.009	0.330 ± 0.009
Relative	8.872 ± 0.274	9.306 ± 0.209	8.943 ± 0.200	8.997 ± 0.233	8.946 ± 0.252	9.533 ± 0.273
Liver						
Absolute	1.660 ± 0.057	1.535 ± 0.036	1.507 ± 0.046	1.589 ± 0.048	1.569 ± 0.034	1.686 ± 0.061
Relative	46.3 ± 0.9	45.8 ± 0.7	44.0 ± 0.6	45.1 ± 0.8	44.1 ± 0.8	48.5 ± 1.1
Lung						
Absolute	0.246 ± 0.013	0.223 ± 0.007	0.227 ± 0.006	0.216 ± 0.009	0.246 ± 0.011	0.226 ± 0.005
Relative	6.939 ± 0.437	6.644 ± 0.189	6.716 ± 0.297	6.127 ± 0.219	6.959 ± 0.375	6.541 ± 0.214
R. Testis						
Absolute	0.122 ± 0.003	0.122 ± 0.003	0.125 ± 0.002	0.128 ± 0.004	0.128 ± 0.002	0.128 ± 0.002
Relative	3.428 ± 0.094	3.653 ± 0.093	3.676 ± 0.089	3.634 ± 0.066	3.634 ± 0.123	3.710 ± 0.095
Thymus						
Absolute	0.034 ± 0.002	0.032 ± 0.002	0.039 ± 0.003	0.037 ± 0.002	0.032 ± 0.003	0.034 ± 0.003
Relative	0.943 ± 0.045	0.963 ± 0.045	1.139 ± 0.087	1.061 ± 0.064	0.885 ± 0.071	0.992 ± 0.090

TABLE H3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame[a]

	0 ppm	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Female						
n	13	15	13	15	15	14
Necropsy body wt	27.51 ± 0.97	28.21 ± 0.79	29.11 ± 1.09	29.39 ± 1.23	27.87 ± 0.81	29.12 ± 0.94
Brain						
Absolute	0.483 ± 0.008	0.488 ± 0.006	0.482 ± 0.008	0.484 ± 0.005	0.488 ± 0.004	0.484 ± 0.004
Relative	17.794 ± 0.634	17.456 ± 0.496	16.754 ± 0.502	16.787 ± 0.570	17.673 ± 0.405	16.821 ± 0.516
Heart						
Absolute	0.147 ± 0.005	0.147 ± 0.004	0.144 ± 0.004	0.141 ± 0.003	0.145 ± 0.004	0.151 ± 0.005
Relative	5.403 ± 0.194	5.251 ± 0.169	4.988 ± 0.148	4.878 ± 0.141	5.223 ± 0.106	5.199 ± 0.162
R. Kidney						
Absolute	0.209 ± 0.007	0.217 ± 0.011	0.215 ± 0.006	0.203 ± 0.005	0.211 ± 0.004	0.220 ± 0.006
Relative	7.656 ± 0.290	7.757 ± 0.454	7.438 ± 0.182	6.999 ± 0.188	7.636 ± 0.193	7.585 ± 0.192
Liver						
Absolute	1.331 ± 0.071	1.281 ± 0.043	1.273 ± 0.045	1.280 ± 0.047	1.302 ± 0.057	1.326 ± 0.052
Relative	48.2 ± 1.5	45.4 ± 0.8	44.1 ± 0.6*	43.7 ± 0.5**	46.6 ± 1.1	45.7 ± 0.8
Lung						
Absolute	0.232 ± 0.016	0.215 ± 0.006	0.211 ± 0.007	0.227 ± 0.011	0.203 ± 0.006	0.219 ± 0.010
Relative	8.387 ± 0.428	7.687 ± 0.252	7.301 ± 0.216	7.794 ± 0.306	7.290 ± 0.133*	7.575 ± 0.328
Thymus						
Absolute	0.038 ± 0.002	0.039 ± 0.003	0.035 ± 0.004	0.040 ± 0.002	0.036 ± 0.003	0.039 ± 0.004
Relative	1.407 ± 0.085	1.374 ± 0.084	1.194 ± 0.126	1.383 ± 0.082	1.272 ± 0.085	1.322 ± 0.113

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

APPENDIX I
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

Procurement and Characterization of Aspartame

Aspartame was obtained from Spectrum Quality Products, Inc. (Gardena, CA), in two shipments of one lot (8415-14-02 RTI) used for the 9-month studies in Tg.AC hemizygous, p53 haploinsufficient, and Cdkn2a deficient mice. Identity and purity analyses were conducted by the analytical chemistry laboratory, Research Triangle Institute (Research Triangle Park, NC), and the study laboratory, BioReliance Corporation (Rockville, MD); the study laboratory also conducted stability analyses. Reports on analyses performed in support of the aspartame studies are on file at the National Institute of Environmental Health Sciences.

Lot 8415-14-02 RTI of the chemical, a white, odorless, crystalline powder, was identified as aspartame by the analytical chemistry laboratory using infrared and proton nuclear magnetic resonance spectroscopy and by the study laboratory using infrared spectroscopy. All spectra were consistent with the structure of aspartame, and the infrared spectra matched a reference spectrum (*Aldrich*, 1981) of aspartame. The infrared and nuclear magnetic resonance spectra are presented in Figures I1 and I2.

The purity of lot 8415-14-02 RTI was determined by the analytical chemistry laboratory using a high-performance liquid chromatography (HPLC) system consisting of a Waters (Milford, MA) HPLC, a Symmetry C_{18} column, 15 cm × 3.9 mm (Waters), photodiode array (205 nm) detection, a mobile phase of 0.02 M pentanesulfonic acid in water, (pH 3.0, adjusted with phosphoric acid) and methanol (70:30), and a flow rate of 1.0 mL/minute (system 1). The study laboratory confirmed purity by conducting major peak comparisons of lot 8415-14-02 RTI with a reference sample of the same lot (stored at less than or equal to –20° C under nitrogen headspace) using an HPLC system similar to system 1 with a Hewlett-Packard (Palo Alto, CA) instrument and a variation in the mobile phase (0.02 M 1-pentanesulfonic acid, sodium salt, in deionized water:methanol; 55:45) (system 2).

For lot 8415-14-02 RTI, HPLC by the analytical chemistry laboratory indicated one major peak and three impurities with a combined area of 0.5% relative to the total peak area. HPLC by the study laboratory indicated a purity of 98.7% for lot 8415-14-02 RTI relative to the reference. The overall purity of lot 8415-14-02 RTI was determined to be greater than 98%.

To ensure stability, the bulk chemical was stored at room temperature, protected from light, in polyethlene bags inside metal drums. The stability of the bulk chemical was monitored during the studies by the study laboratory using HPLC system 2. No degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared every 2 weeks by mixing aspartame with feed (Table I1). A premix was prepared by hand and then blended with additional feed in a Patterson-Kelly twin-shell blender for 15 minutes using an intensifier bar for the initial five minutes. Formulations were stored in doubled polyethylene bags at 2° C to 8° C, protected from light, for up to 28 days.

Homogeneity and stability studies of dose formulations containing 1,000, 10,000, and 50,000 ppm aspartame were performed by the analytical chemistry laboratory using HPLC by system 1 with a variation in the mobile phase ratio (60:40). Homogeneity studies of formulations containing 3,125 and 50,000 ppm aspartame were performed by the study laboratory using HPLC (system 2). Homogeneity was confirmed, and stability was confirmed for up to 28 days at –20° C and 5° C for dose formulations stored in doubled polyethylene bags. There was no significant loss of aspartame from NTP-2000 feed formulations exposed to air and light for 7 days.

Periodic analyses of the dose formulations of aspartame were conducted by the study laboratory using HPLC by system 2. During the 9-month studies, the dose formulations were analyzed seven times; all (35/35) of the dose formulations analyzed were within 10% of the target concentrations (Table I2). Animal room samples of these dose formulations were also analyzed, and 27 of 35 animal room samples were within 10% of the target concentrations (Table I2). Dose formulations were stored refrigerated, protected from light, in doubled polyethylene bags for up to 28 days.

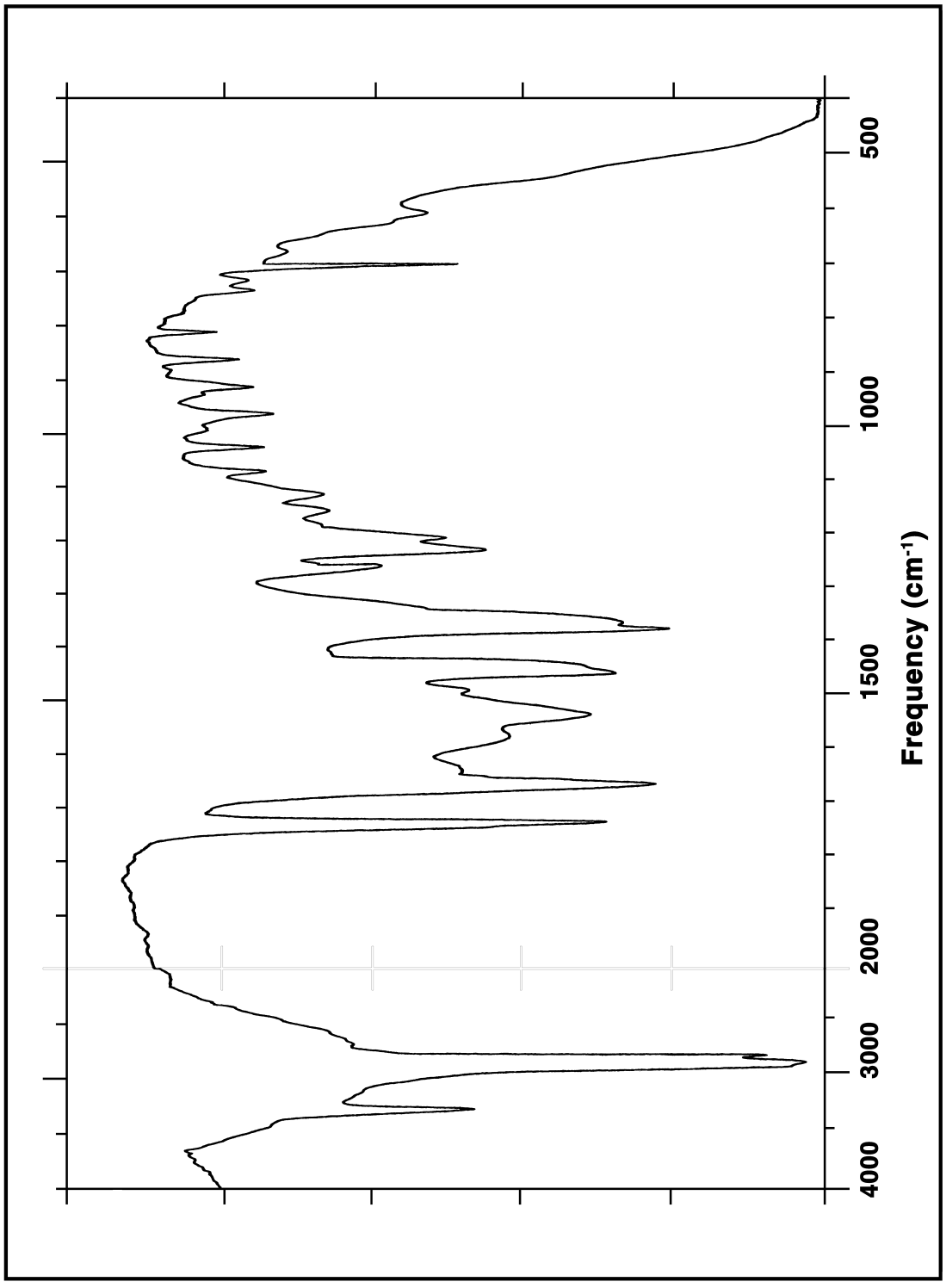


FIGURE I1
Infrared Absorption Spectrum of Aspartame

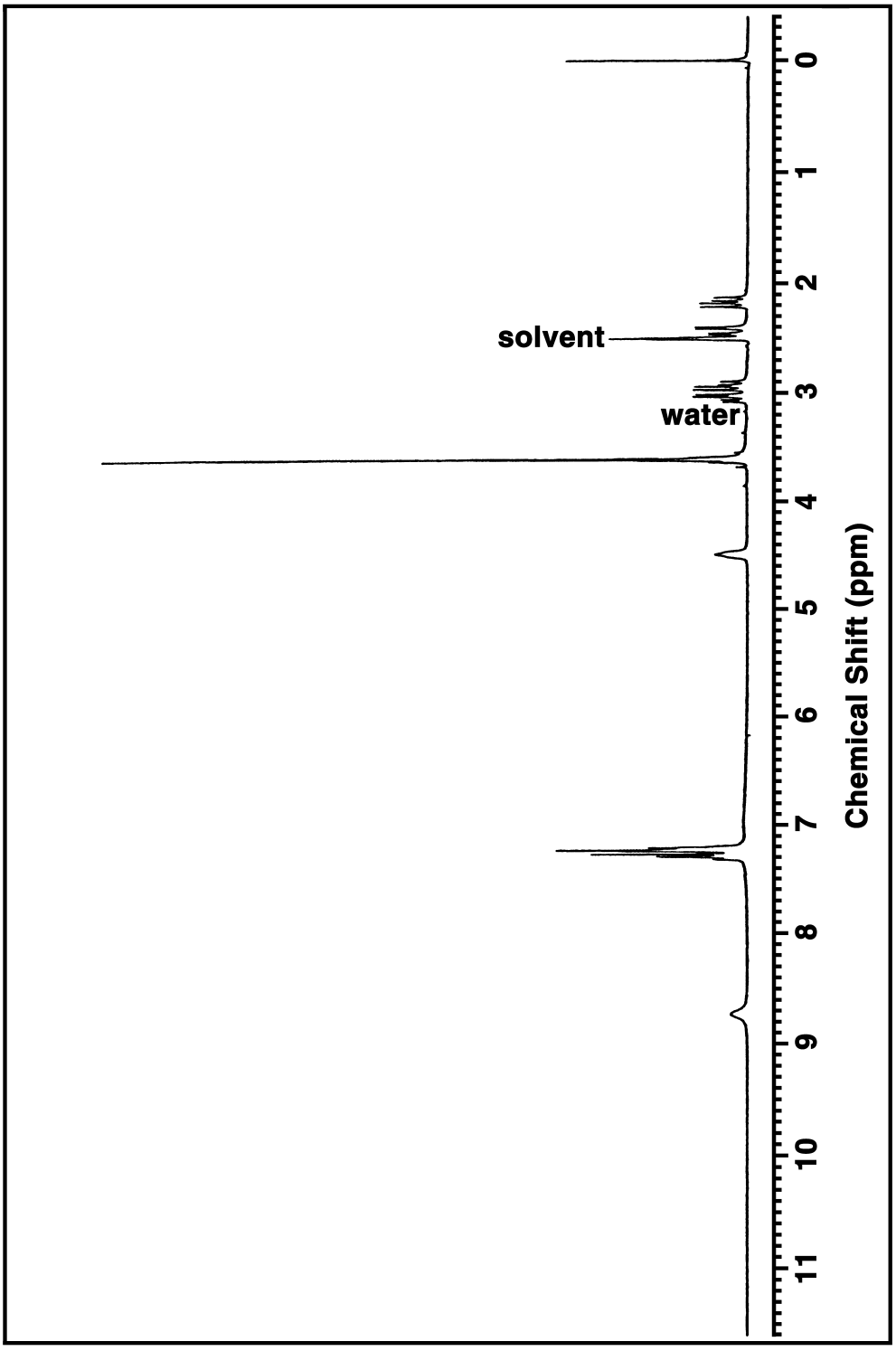


FIGURE I2
Proton Nuclear Magnetic Resonance Spectrum of Aspartame

TABLE I1
Preparation and Storage of Dose Formulations in the 9-Month Feed Studies of Aspartame

Preparation
A premix of feed and aspartame was prepared, then layered into the remaining feed and blended in a Patterson-Kelley twin-shell blender with the intensifier bar on for 5 minutes and off for 10 minutes. The dose formulations were prepared every 2 weeks.

Chemical Lot Number
8415-14-02 RTI

Maximum Storage Time
28 days

Storage Conditions
Stored in doubled polyethylene bags at 2° to 8° C, protected from light

Study Laboratory
BioReliance Corporation (Rockville, MD)

TABLE I2
Results of Analyses of Dose Formulations Administered to Tg.AC Hemizygous Mice, p53 Haploinsufficient Mice, and Cdkn2a Deficient Mice in the 9-Month Feed Studies of Aspartame

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
October 5, 1999	October 5, 1999	3,125	3,090	–1
		6,250	6,290	+1
		12,500	12,400	–1
		25,000	25,200	+1
		50,000	51,200	+2
	October 26, 1999[b]	3,125	2,610	–16
		6,250	5,480	–12
		12,500	11,300	–10
		25,000	23,100	–8
		50,000	45,700	–9
November 30, 1999	November 30, 1999	3,125	3,140	0
		6,250	6,210	–1
		12,500	12,700	+2
		25,000	24,700	–1
		50,000	48,500	–3
	December 28, 1999[b]	3,125	2,880	–8
		6,250	5,380	–14
		12,500	11,400	–9
		25,000	22,600	–10
		50,000	46,500	–7
February 23, 2000	February 23, 2000	3,125	3,370	+8
		6,250	6,610	+6
		12,500	13,300	+6
		25,000	26,200	+5
		50,000	51,200	+2
	March 15, 2000[b]	3,125	3,030	–3
		6,250	6,030	–4
		12,500	12,200	–2
		25,000	24,400	–2
		50,000	49,300	–1
April 4, 2000	April 4, 2000	3,125	3,130	0
		6,250	6,430	+3
		12,500	12,800	+2
		25,000	25,200	+1
		50,000	50,400	+1
	April 26, 2000[b]	3,125	2,550	–18
		6,250	5,450	–13
		12,500	10,700	–14
		25,000	20,900	–16
		50,000	46,000	–8

TABLE I2
Results of Analyses of Dose Formulations Administered to Tg.AC Hemizygous Mice, p53 Haploinsufficient Mice, and Cdkn2a Deficient Mice in the 9-Month Feed Studies of Aspartame

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
(continued)				
May 16, 2000	May 16, 2000	3,125	2,820	–10
		6,250	5,790	–7
		12,500	12,400	–1
		25,000	27,300	+9
		50,000	52,800	+6
	June 14, 2000[b]	3,125	2,700	–14
		6,250	5,610	–10
		12,500	11,700	–6
		25,000	22,700	–9
		50,000	48,500	–3
June 27, 2000	June 27, 2000	3,125	3,310	+6
		6,250	6,420	+3
		12,500	13,100	+5
		25,000	26,000	+4
		50,000	51,000	+2
	August 14, 2000[b]	3,125	2,890	–8
		6,250	5,980	–4
		12,500	12,600	+1
		25,000	24,700	–1
		50,000	51,800	+4
July 11, 2000	July 11, 2000	3,125	3,320	+6
		6,250	6,590	+5
		12,500	13,000	+4
		25,000	25,700	+3
		50,000	49,800	0
	August 14, 2000[b]	3,125	2,970	–5
		6,250	5,930	–5
		12,500	11,600	–7
		25,000	24,000	–4
		50,000	47,100	–6

[a] Results of duplicate analyses
[b] Animal room samples

APPENDIX J
FEED AND COMPOUND CONSUMPTION IN THE 9-MONTH FEED STUDIES OF ASPARTAME

TABLE J1
Feed and Compound Consumption by Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame

Week	0 ppm		3,125 ppm			6,250 ppm			12,500 ppm		
	Feed (g)[a]	Body Weight (g)	Feed (g)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g)	Body Weight (g)	Dose (mg/kg)	Feed (g)	Body Weight (g)	Dose (mg/kg)
2	4.5	21.5	4.2	21.3	615	4.2	20.1	1,304	4.3	20.3	2,631
3	4.1	21.9	4.0	22.0	572	4.4	21.8	1,267	4.3	21.2	2,510
4	4.5	22.6	4.5	23.2	601	4.4	22.7	1,204	4.3	22.3	2,432
5	4.5	23.8	4.3	23.7	570	4.5	23.6	1,190	4.3	23.1	2,312
6	4.2	24.6	4.1	23.2	552	4.4	24.6	1,129	4.2	23.5	2,244
7	4.5	25.2	4.2	24.8	531	4.4	25.7	1,081	4.0	23.9	2,075
8	4.5	26.0	4.2	25.0	523	4.3	25.8	1,031	4.2	24.8	2,125
9	4.4	26.3	4.3	25.9	516	4.4	26.8	1,019	4.4	25.6	2,125
10	4.3	27.4	4.3	26.4	513	4.3	26.7	1,007	4.3	26.1	2,059
11	4.3	28.2	4.4	26.7	513	4.5	27.5	1,032	4.6	26.8	2,125
12	4.4	28.5	4.1	26.2	487	4.5	28.0	1,015	4.3	26.6	2,001
13	4.3	28.8	4.4	27.4	496	4.5	28.3	1,006	4.2	27.0	1,923
14	4.2	29.0	4.2	27.1	479	4.2	27.8	949	4.2	27.2	1,933
15	4.2	29.5	4.5	28.5	490	4.5	29.2	966	4.2	28.0	1,872
16	4.2	29.5	4.1	27.8	460	4.3	28.8	928	4.3	27.9	1,923
17	4.2	29.2	4.3	27.9	486	4.3	29.1	930	4.2	28.3	1,876
18	4.1	29.5	4.4	28.6	478	4.3	29.2	928	4.3	28.3	1,881
19	4.1	29.1	4.4	29.4	471	4.4	29.7	926	4.2	28.2	1,845
20	4.4	29.7	4.1	29.0	446	4.1	29.9	864	4.2	28.6	1,851
21	4.3	30.2	4.4	29.6	469	4.5	30.3	921	4.3	29.0	1,874
22	4.2	30.4	4.2	29.1	447	4.0	29.7	847	4.3	29.0	1,873
23	4.3	30.8	4.2	29.1	450	4.5	29.9	948	4.4	29.2	1,892
24	4.2	30.7	4.2	28.8	451	4.2	30.1	877	4.3	29.9	1,780
25	4.4	31.1	4.3	29.1	461	4.3	30.5	890	4.5	30.0	1,856
26	4.0	30.8	4.3	29.4	460	4.4	30.7	902	4.2	30.0	1,758
27	4.3	31.3	4.4	29.4	466	4.2	30.7	861	4.1	29.7	1,738
28	4.1	31.5	4.3	29.3	458	4.2	30.5	869	4.2	29.7	1,785
29	4.2	31.4	4.2	28.8	460	4.0	30.0	840	4.1	30.1	1,719
30	4.4	31.8	4.6	30.2	472	4.5	30.0	931	4.4	30.7	1,779
31	4.5	31.5	4.6	30.4	471	4.7	30.6	968	4.6	30.5	1,869
32	4.3	31.1	4.6	30.6	470	4.8	30.9	961	4.4	30.1	1,830
33	4.4	31.1	4.8	30.6	494	4.7	30.8	960	4.6	30.0	1,918
34	4.5	31.3	4.7	30.5	482	4.6	31.2	925	4.8	30.3	1,965
35	4.5	31.6	4.6	30.6	470	4.6	30.7	928	4.7	30.8	1,914
36	4.6	32.0	4.6	30.6	467	4.8	31.7	952	4.8	31.3	1,896
37	4.4	32.0	4.5	30.1	464	4.7	31.9	918	4.5	30.9	1,832
38	4.3	31.7	4.7	30.9	475	4.4	31.6	878	4.3	31.2	1,739
39	4.2	31.8	4.6	31.1	461	4.6	31.8	898	4.5	31.3	1,796
40	4.4	32.8	4.3	31.3	428	4.7	32.1	907	4.5	31.2	1,802
Mean for weeks											
2-13	4.4	25.4	4.2	24.7	541	4.4	25.1	1,107	4.3	24.3	2,214
14-40	4.3	30.8	4.4	29.5	466	4.4	30.3	914	4.4	29.7	1,844

TABLE J1
Feed and Compound Consumption by Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame

Week	25,000 ppm			50,000 ppm		
	Feed (g)	Body Weight (g)	Dose (mg/kg)	Feed (g)	Body Weight (g)	Dose (mg/kg)
2	4.3	21.7	5,005	4.1	20.8	9,871
3	4.4	22.1	4,998	4.2	22.0	9,590
4	4.5	23.3	4,808	4.3	22.7	9,448
5	4.4	23.9	4,642	4.3	23.6	9,028
6	4.5	25.0	4,540	4.1	23.8	8,695
7	4.4	25.4	4,375	4.2	24.9	8,394
8	4.5	25.9	4,332	4.1	24.6	8,247
9	4.3	26.1	4,168	4.3	25.5	8,340
10	4.3	26.4	4,027	4.3	26.2	8,210
11	4.5	27.5	4,056	4.4	26.8	8,178
12	4.2	26.7	3,968	4.3	27.0	8,059
13	4.7	27.9	4,170	4.4	27.4	7,993
14	4.5	28.1	3,985	4.4	27.6	7,958
15	4.4	28.5	3,876	4.2	28.1	7,465
16	4.4	28.4	3,841	4.1	27.8	7,396
17	4.5	29.0	3,879	4.2	28.6	7,348
18	4.4	28.8	3,779	4.2	28.6	7,423
19	4.5	29.3	3,840	4.3	28.4	7,611
20	4.2	29.9	3,479	4.1	28.9	7,128
21	4.2	29.6	3,584	4.2	29.4	7,092
22	4.4	29.4	3,724	3.9	28.5	6,829
23	4.5	29.7	3,766	4.2	28.4	7,373
24	4.4	30.0	3,635	4.4	27.9	7,866
25	4.5	29.6	3,820	4.0	28.0	7,176
26	4.5	30.3	3,724	4.0	27.2	7,385
27	4.5	30.5	3,668	4.1	29.0	7,075
28	4.6	30.7	3,713	4.2	29.7	7,085
29	4.5	30.9	3,622	4.1	30.0	6,796
30	4.6	31.1	3,669	4.3	30.9	6,998
31	4.8	31.0	3,854	4.6	30.9	7,479
32	4.7	31.3	3,756	4.3	31.0	6,938
33	4.5	31.1	3,591	4.5	31.2	7,174
34	4.8	30.8	3,862	4.3	30.5	7,085
35	4.4	30.3	3,603	4.3	31.1	6,951
36	4.4	29.8	3,704	4.5	31.6	7,044
37	4.2	29.7	3,536	4.2	31.2	6,662
38	4.7	30.3	3,881	4.2	31.3	6,790
39	4.8	31.7	3,817	4.1	31.2	6,564
40	4.6	31.2	3,667	4.5	31.7	7,023
Mean for weeks						
2-13	4.4	25.2	4,424	4.2	24.6	8,671
14-40	4.5	30.0	3,736	4.2	29.6	7,175

[a] Grams of feed consumed per animal per day
[b] Milligrams of aspartame consumed per kilogram body weight per day

TABLE J2
Feed and Compound Consumption by Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame

Week	0 ppm		3,125 ppm			6,250 ppm			12,500 ppm		
	Feed (g)[a]	Body Weight (g)	Feed (g)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g)	Body Weight (g)	Dose (mg/kg)	Feed (g)	Body Weight (g)	Dose (mg/kg)
2	4.2	18.1	4.2	18.0	724	4.1	17.7	1,432	4.1	17.4	2,918
3	4.0	18.5	4.1	18.9	682	3.8	18.3	1,285	3.8	17.8	2,649
4	4.5	19.5	4.2	19.7	669	4.1	19.5	1,306	4.0	18.8	2,643
5	4.1	19.5	4.0	20.1	618	3.8	19.7	1,206	3.9	19.7	2,488
6	4.2	19.7	4.1	20.3	634	4.1	20.4	1,269	4.1	19.3	2,658
7	4.1	20.1	4.0	21.4	581	3.9	21.3	1,155	4.1	20.7	2,458
8	4.1	20.5	3.8	20.9	566	3.7	20.6	1,118	4.0	20.6	2,404
9	4.2	21.1	4.0	21.9	570	3.9	21.5	1,134	4.1	21.3	2,397
10	4.0	21.6	4.1	22.2	574	4.0	21.5	1,156	4.1	21.9	2,327
11	3.8	21.7	4.0	22.4	559	4.2	22.5	1,165	4.0	21.9	2,308
12	4.1	22.4	4.1	22.6	561	4.1	21.9	1,166	4.1	21.9	2,326
13	4.1	22.5	4.1	22.6	568	3.9	22.0	1,117	4.2	22.3	2,372
14	4.0	22.4	4.1	22.9	564	4.3	23.0	1,158	4.1	22.5	2,257
15	3.7	22.3	3.6	22.5	494	3.9	23.3	1,057	4.1	22.4	2,283
16	3.8	22.6	4.2	23.3	567	4.0	23.4	1,056	4.0	22.5	2,224
17	4.0	22.6	3.9	23.1	528	3.8	23.2	1,027	4.1	22.5	2,263
18	4.0	22.6	4.1	23.3	544	4.2	23.4	1,112	4.1	22.9	2,228
19	3.8	23.0	4.0	23.3	537	4.2	23.8	1,110	4.1	23.4	2,188
20	4.0	23.1	3.9	23.1	523	3.9	23.7	1,024	3.8	23.0	2,090
21	4.1	23.9	4.1	24.2	535	4.3	24.7	1,084	3.9	23.3	2,065
22	4.1	23.7	4.1	24.3	527	4.3	24.6	1,088	3.9	22.9	2,147
23	4.6	24.4	4.0	24.6	513	4.2	24.6	1,068	4.1	23.1	2,222
24	4.3	24.4	4.0	24.1	515	4.1	24.8	1,044	3.8	23.1	2,033
25	4.0	24.4	4.2	25.2	525	4.2	25.0	1,037	4.0	24.0	2,071
26	4.3	24.9	4.0	25.6	492	4.2	25.0	1,053	4.4	23.9	2,281
27	4.2	25.1	4.1	25.6	498	3.9	24.9	969	4.0	23.5	2,126
28	4.0	25.3	3.8	25.2	475	4.3	25.1	1,061	4.2	25.0	2,107
29	4.2	25.3	3.9	25.4	479	3.8	24.9	955	3.8	24.9	1,928
30	4.6	26.0	3.8	25.0	481	4.3	25.6	1,039	4.5	25.4	2,209
31	4.5	25.8	4.2	25.1	526	4.5	26.3	1,065	4.3	25.1	2,139
32	4.4	25.1	4.4	25.1	544	4.3	26.1	1,036	4.6	25.3	2,246
33	4.3	25.6	4.4	25.1	554	4.6	26.4	1,091	4.4	25.0	2,219
34	4.3	25.6	4.6	25.1	573	4.8	26.6	1,132	4.5	25.4	2,202
35	4.1	25.4	4.5	26.0	540	4.3	26.9	1,007	4.4	26.1	2,115
36	4.6	26.1	4.2	25.7	512	4.4	27.8	998	4.3	26.3	2,026
37	4.4	25.9	3.8	25.4	472	4.3	27.6	982	4.3	25.8	2,064
38	4.5	26.2	4.3	26.2	507	4.1	27.0	945	4.2	26.0	2,000
39	4.3	26.5	4.0	26.4	474	4.1	27.6	932	4.3	26.4	2,025
40	4.3	26.7	4.1	25.4	500	4.3	26.9	1,000	4.4	26.5	2,055
Mean for weeks											
2-13	4.1	20.4	4.1	20.9	609	4.0	20.6	1,209	4.0	20.3	2,496
14-40	4.2	24.6	4.1	24.7	518	4.2	25.3	1,042	4.2	24.3	2,141

TABLE J2
Feed and Compound Consumption by Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame

Week	25,000 ppm			50,000 ppm		
	Feed (g)	Body Weight (g)	Dose (mg/kg)	Feed (g)	Body Weight (g)	Dose (mg/kg)
2	4.2	18.1	5,774	3.9	17.8	10,879
3	3.8	17.6	5,388	3.8	17.9	10,617
4	4.1	19.6	5,255	4.0	19.6	10,200
5	3.9	20.1	4,804	3.8	20.3	9,293
6	3.6	20.2	4,408	3.8	20.2	9,351
7	4.1	20.8	4,911	3.8	21.3	9,017
8	4.1	21.1	4,848	3.6	20.7	8,771
9	4.0	21.6	4,578	4.0	21.8	9,301
10	3.9	21.9	4,461	3.9	22.4	8,760
11	3.8	22.5	4,271	3.8	22.7	8,466
12	3.8	21.7	4,424	3.9	23.2	8,460
13	4.4	23.2	4,737	4.0	23.1	8,756
14	4.4	22.9	4,768	4.1	23.1	8,832
15	4.2	23.6	4,495	3.8	23.5	8,133
16	3.9	23.4	4,183	4.2	24.5	8,461
17	4.1	23.8	4,262	3.9	24.4	7,892
18	4.1	23.7	4,273	3.9	24.5	8,036
19	4.1	23.6	4,320	4.2	24.9	8,436
20	4.1	23.7	4,302	4.0	25.9	7,669
21	4.0	24.1	4,152	3.9	26.0	7,512
22	4.2	24.2	4,328	3.8	25.7	7,319
23	4.1	23.9	4,301	4.1	25.7	7,991
24	4.1	24.3	4,212	4.3	26.1	8,163
25	4.1	24.4	4,190	3.9	26.2	7,483
26	4.4	24.7	4,439	3.8	26.1	7,339
27	4.1	24.9	4,145	3.8	26.3	7,318
28	4.2	25.1	4,151	3.9	27.3	7,214
29	4.1	25.0	4,140	3.9	27.5	7,156
30	4.1	24.8	4,119	3.9	27.2	7,180
31	4.1	24.4	4,220	4.2	27.9	7,521
32	4.2	24.3	4,370	4.1	28.0	7,251
33	4.2	24.6	4,282	4.3	27.8	7,671
34	4.2	24.7	4,243	4.1	27.9	7,433
35	4.4	25.3	4,292	4.1	27.7	7,364
36	3.8	25.0	3,827	3.9	27.8	7,086
37	3.8	24.7	3,866	4.1	27.2	7,545
38	3.9	24.6	3,959	4.2	28.1	7,425
39	4.0	24.8	4,042	4.0	28.0	7,132
40	4.4	26.5	4,195	4.2	28.3	7,456
Mean for weeks						
2-13	4.0	20.7	4,822	3.9	20.9	9,323
14-40	4.1	24.4	4,225	4.0	26.4	7,630

[a] Grams of feed consumed per animal per day
[b] Milligrams of aspartame consumed per kilogram body weight per day

TABLE J3
Feed and Compound Consumption by Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame

Week	0 ppm		3,125 ppm			6,250 ppm			12,500 ppm		
	Feed (g)[a]	Body Weight (g)	Feed (g)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g)	Body Weight (g)	Dose (mg/kg)	Feed (g)	Body Weight (g)	Dose (mg/kg)
2	4.1	22.0	4.5	21.8	639	4.3	21.4	1,242	4.0	20.9	2,395
3	4.2	22.6	4.1	22.3	581	4.2	22.2	1,195	4.0	21.6	2,318
4	4.1	22.8	4.3	23.0	588	4.4	22.4	1,218	4.1	22.5	2,296
5	4.3	23.5	4.3	23.6	568	4.2	22.9	1,151	4.1	22.9	2,254
6	4.2	24.0	4.3	24.1	563	4.3	23.7	1,130	3.9	23.4	2,106
7	4.3	24.8	4.3	24.7	549	4.2	24.2	1,074	4.0	23.9	2,085
8	4.3	25.7	4.3	25.5	528	4.1	24.5	1,045	4.1	24.6	2,104
9	4.1	26.1	4.3	25.9	522	4.0	24.9	1,013	3.9	24.9	1,946
10	4.2	26.6	4.5	26.2	537	4.5	25.3	1,120	3.9	25.6	1,912
11	4.0	27.0	4.6	26.7	533	4.5	25.5	1,110	4.1	26.0	1,950
12	4.1	27.1	4.6	27.2	532	4.2	25.9	1,014	4.3	26.5	2,027
13	4.1	27.6	4.4	27.6	496	4.3	26.8	1,010	4.0	26.8	1,866
14	4.3	28.0	4.2	27.8	471	4.3	27.3	992	4.1	27.3	1,864
15	4.2	28.3	4.4	28.2	492	4.5	27.4	1,026	4.0	27.5	1,814
16	4.4	28.6	4.4	28.4	480	4.0	27.0	931	4.1	28.1	1,839
17	4.4	29.1	4.5	28.8	493	4.4	27.8	983	4.2	28.2	1,869
18	4.3	29.4	4.5	29.2	480	4.2	28.7	924	4.4	28.9	1,892
19	4.3	29.8	4.3	29.4	452	4.1	29.0	884	3.9	28.9	1,692
20	4.2	30.1	4.1	29.3	441	4.1	29.1	875	3.8	28.8	1,647
21	4.1	30.3	4.2	29.6	449	4.2	29.7	885	3.9	29.3	1,671
22	4.0	30.0	4.3	29.5	455	4.1	29.5	861	4.1	29.6	1,731
23	4.1	30.8	4.3	29.0	459	4.2	29.8	876	3.7	29.0	1,596
24	4.1	30.6	4.3	29.6	451	4.2	29.8	883	3.7	29.0	1,578
25	3.9	30.4	4.3	29.6	457	4.2	29.8	873	4.0	29.2	1,693
26	4.1	30.9	4.4	30.0	453	4.2	29.7	873	4.0	29.5	1,688
27	4.0	30.8	4.1	29.8	435	4.1	30.0	851	4.0	29.6	1,694
28	4.3	31.0	4.2	29.9	435	4.0	29.9	829	3.9	29.7	1,653
29	4.2	31.2	4.2	30.2	438	4.1	29.9	847	4.0	29.8	1,673
30	4.5	31.8	4.4	30.6	454	4.3	29.8	896	4.2	30.2	1,746
31	4.4	32.3	4.4	31.0	445	4.3	30.3	884	4.2	30.0	1,752
32	4.5	32.6	4.3	31.0	434	4.3	30.6	868	4.1	29.9	1,723
33	4.3	32.4	4.3	30.9	437	4.3	30.3	879	4.2	29.8	1,745
34	4.3	32.3	4.3	30.6	443	4.5	30.5	917	4.1	29.9	1,710
35	4.6	32.2	4.5	30.7	453	4.2	30.2	868	4.5	29.7	1,907
36	4.4	32.4	4.4	30.9	445	4.2	30.1	875	4.2	29.9	1,754
37	4.2	31.6	4.4	30.6	445	4.3	30.1	897	4.2	29.7	1,761
38	4.3	31.9	4.4	30.9	447	4.4	30.1	905	4.1	29.3	1,746
39	4.1	31.9	4.2	30.4	432	4.4	29.6	922	3.9	29.4	1,653
40	4.2	31.3	4.3	30.3	448	4.3	29.7	911	4.5	29.8	1,872
Mean for weeks											
2-13	4.2	25.0	4.4	24.9	553	4.3	24.1	1,110	4.0	24.1	2,105
14-40	4.2	30.8	4.3	29.9	453	4.2	29.5	897	4.1	29.3	1,739

TABLE J3
Feed and Compound Consumption by Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame

Week	25,000 ppm			50,000 ppm		
	Feed (g)	Body Weight (g)	Dose (mg/kg)	Feed (g)	Body Weight (g)	Dose (mg/kg)
2	4.2	21.5	4,832	3.9	21.4	9,070
3	4.1	21.8	4,696	3.7	21.6	8,475
4	4.2	22.8	4,655	3.8	22.5	8,556
5	4.3	23.3	4,593	4.0	23.0	8,662
6	4.1	23.9	4,241	4.0	23.3	8,474
7	4.1	24.6	4,187	4.0	24.1	8,223
8	4.1	24.7	4,120	4.1	24.5	8,320
9	4.2	25.4	4,160	3.8	24.8	7,634
10	4.4	26.1	4,268	4.0	25.5	7,810
11	4.1	26.6	3,859	4.0	25.8	7,796
12	4.7	27.3	4,266	4.5	26.5	8,551
13	4.3	27.3	3,914	4.1	27.0	7,562
14	4.3	28.1	3,865	4.1	27.5	7,453
15	4.1	28.1	3,652	3.9	27.5	7,061
16	4.4	28.4	3,887	4.2	27.8	7,460
17	4.5	29.0	3,842	4.1	28.1	7,288
18	4.4	29.3	3,713	4.3	28.7	7,453
19	4.1	29.4	3,460	4.1	29.2	6,937
20	4.3	30.2	3,559	3.9	29.5	6,627
21	4.2	30.4	3,441	3.8	29.5	6,350
22	3.9	30.0	3,240	3.8	29.0	6,538
23	3.9	29.9	3,272	3.8	29.1	6,500
24	4.0	29.7	3,404	3.8	29.1	6,520
25	3.8	29.2	3,275	3.7	29.1	6,276
26	4.3	29.5	3,658	4.0	29.3	6,747
27	4.0	29.6	3,395	3.9	29.4	6,667
28	3.9	28.8	3,357	3.9	29.7	6,523
29	4.1	29.0	3,508	3.9	29.7	6,613
30	4.2	29.3	3,578	4.1	29.7	6,861
31	4.2	29.4	3,578	4.2	30.2	6,970
32	4.2	29.5	3,541	4.0	30.3	6,571
33	4.3	29.7	3,644	4.1	30.5	6,674
34	4.6	30.3	3,811	4.0	30.2	6,611
35	4.3	30.1	3,541	4.2	30.2	6,964
36	4.3	30.1	3,533	4.1	30.1	6,869
37	4.0	29.7	3,383	4.0	30.1	6,654
38	4.3	29.9	3,583	4.2	30.0	6,971
39	4.4	30.1	3,642	4.1	29.9	6,798
40	4.3	30.1	3,526	4.1	29.9	6,790
Mean for weeks						
2-13	4.2	24.6	4,316	4.0	24.2	8,261
14-40	4.2	29.5	3,551	4.0	29.4	6,805

[a] Grams of feed consumed per animal per day
[b] Milligrams of aspartame consumed per kilogram body weight per day

TABLE J4
Feed and Compound Consumption by Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame

Week	0 ppm		3,125 ppm			6,250 ppm			12,500 ppm		
	Feed (g)[a]	Body Weight (g)	Feed (g)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g)	Body Weight (g)	Dose (mg/kg)	Feed (g)	Body Weight (g)	Dose (mg/kg)
2	3.9	18.5	4.1	18.1	706	4.0	18.2	1,378	4.2	18.1	2,898
3	4.4	19.6	4.3	19.2	695	4.0	18.9	1,313	4.1	18.7	2,724
4	4.7	20.0	4.8	20.2	737	4.5	19.9	1,407	4.7	19.9	2,938
5	4.9	20.8	4.7	20.5	714	4.7	20.5	1,444	4.8	20.3	2,991
6	4.8	21.0	4.7	20.5	722	4.8	21.2	1,404	4.6	20.6	2,798
7	5.2	20.7	4.7	20.7	707	4.8	20.5	1,465	4.9	20.6	2,950
8	5.0	21.5	4.5	20.9	676	5.0	21.4	1,451	4.8	21.3	2,827
9	4.8	21.7	4.7	21.5	689	5.0	22.0	1,414	5.0	21.4	2,934
10	5.3	22.1	5.0	21.8	714	4.9	22.0	1,380	4.8	22.2	2,699
11	5.6	22.1	4.8	22.0	687	5.3	22.4	1,474	5.1	22.3	2,830
12	5.1	22.0	4.8	22.0	682	4.4	22.6	1,222	5.1	22.5	2,828
13	5.1	22.2	4.9	22.6	683	4.7	23.4	1,261	4.7	22.5	2,635
14	4.5	22.6	4.4	23.0	592	4.2	23.5	1,106	4.5	22.9	2,448
15	4.3	22.9	4.6	23.2	621	4.3	23.9	1,134	4.2	22.9	2,267
16	4.7	22.7	5.0	23.3	670	4.4	24.0	1,146	5.1	23.3	2,735
17	4.9	23.1	4.8	23.4	640	4.9	24.0	1,272	4.7	23.6	2,493
18	4.9	23.3	4.8	23.4	640	4.5	24.4	1,153	5.6	24.4	2,852
19	4.6	23.6	4.5	23.5	598	4.3	24.8	1,074	4.8	24.6	2,440
20	4.6	23.7	4.3	23.8	569	4.2	24.8	1,051	4.5	25.1	2,227
21	4.0	23.7	4.2	23.5	563	4.3	25.1	1,080	4.4	25.1	2,195
22	4.9	24.1	4.4	23.7	584	4.6	25.0	1,139	4.9	25.1	2,436
23	4.8	24.0	4.7	23.4	623	4.7	25.3	1,164	4.3	24.9	2,165
24	4.8	24.2	4.7	23.8	613	4.5	25.5	1,112	4.5	24.7	2,295
25	4.8	24.2	4.7	23.6	617	4.6	25.4	1,120	4.7	24.7	2,382
26	4.5	24.3	4.6	23.7	607	4.8	25.7	1,156	4.8	25.1	2,418
27	4.7	23.9	4.3	23.4	579	4.5	25.7	1,090	4.4	25.1	2,218
28	4.9	24.5	4.4	23.4	590	4.6	25.8	1,109	4.8	25.1	2,376
29	4.6	24.2	4.6	23.4	616	4.7	25.8	1,144	4.7	25.1	2,340
30	4.7	25.0	4.5	24.1	579	4.5	25.5	1,102	4.6	25.8	2,238
31	4.4	25.2	4.3	24.1	555	4.4	25.7	1,068	4.4	26.0	2,115
32	4.4	25.3	4.4	24.4	559	4.3	25.8	1,033	4.4	25.8	2,141
33	4.7	24.9	4.4	24.0	572	4.5	25.8	1,093	4.7	25.9	2,252
34	4.6	25.2	4.6	24.5	589	4.3	25.9	1,032	4.4	25.9	2,133
35	4.8	25.2	4.5	24.7	564	6.8	25.8	1,650	5.3	25.8	2,572
36	4.6	25.4	4.5	24.7	569	4.7	26.4	1,117	4.4	26.0	2,131
37	4.7	25.3	4.5	24.6	567	4.5	26.3	1,074	4.8	26.0	2,291
38	4.9	25.7	4.5	24.4	580	4.8	26.6	1,135	4.9	26.0	2,336
39	4.4	25.7	4.6	24.4	585	4.5	26.5	1,066	4.4	25.8	2,134
40	4.7	26.1	4.5	24.3	580	4.6	26.2	1,086	4.6	25.4	2,252
Mean for weeks											
2-13	4.9	21.0	4.7	20.8	701	4.7	21.1	1,384	4.7	20.9	2,838
14-40	4.6	24.4	4.5	23.8	593	4.6	25.4	1,130	4.7	25.0	2,329

TABLE J4
Feed and Compound Consumption by Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame

Week	25,000 ppm			50,000 ppm		
	Feed (g)	Body Weight (g)	Dose (mg/kg)	Feed (g)	Body Weight (g)	Dose (mg/kg)
2	4.1	18.2	5,686	3.8	17.7	10,687
3	4.2	19.0	5,551	4.0	18.6	10,601
4	4.4	20.1	5,428	4.0	19.3	10,387
5	4.5	20.1	5,589	4.2	19.9	10,482
6	4.4	20.6	5,344	4.3	20.3	10,630
7	4.7	20.8	5,670	4.6	19.9	11,459
8	4.5	21.0	5,347	4.5	21.3	10,536
9	4.9	21.7	5,581	4.3	21.3	9,994
10	4.9	21.7	5,648	3.9	21.9	8,969
11	5.1	22.2	5,702	4.6	21.8	10,565
12	4.6	22.3	5,157	4.8	21.9	10,939
13	4.8	22.5	5,349	4.6	22.3	10,385
14	4.6	22.9	5,026	4.3	22.6	9,436
15	5.1	23.6	5,416	4.6	22.9	10,064
16	4.7	23.4	4,989	5.0	23.1	10,709
17	4.7	22.9	5,080	4.0	23.0	8,713
18	4.8	23.3	5,140	4.8	23.5	10,290
19	4.6	23.6	4,891	4.6	23.6	9,754
20	4.4	23.8	4,643	4.5	24.1	9,340
21	4.3	24.0	4,473	4.3	24.3	8,764
22	4.5	23.7	4,702	4.3	24.0	8,868
23	4.7	23.7	4,915	4.1	23.6	8,710
24	4.5	23.8	4,686	4.6	24.2	9,482
25	4.6	23.5	4,888	4.4	24.1	9,102
26	4.8	24.3	4,956	4.5	24.4	9,152
27	4.4	24.0	4,540	4.5	24.2	9,354
28	4.6	23.9	4,761	4.5	24.3	9,267
29	4.5	24.0	4,676	4.3	24.4	8,775
30	4.7	24.2	4,851	4.2	24.3	8,690
31	4.6	24.5	4,712	4.3	24.7	8,720
32	4.2	24.3	4,273	4.5	24.6	9,187
33	4.7	24.5	4,795	4.6	25.0	9,121
34	4.5	24.9	4,525	4.5	24.6	9,199
35	5.1	24.9	5,113	4.6	24.5	9,396
36	4.5	25.1	4,444	4.3	24.9	8,708
37	4.6	25.0	4,578	4.3	24.8	8,746
38	4.8	25.4	4,719	4.5	25.1	9,009
39	4.9	25.4	4,844	4.5	25.0	8,939
40	4.7	25.3	4,617	4.7	25.1	9,338
Mean for weeks						
2-13	4.6	20.9	5,504	4.3	20.5	10,469
14-40	4.6	24.2	4,787	4.5	24.2	9,216

[a] Grams of feed consumed per animal per day
[b] Milligrams of aspartame consumed per kilogram body weight per day

TABLE J5
Feed and Compound Consumption by Male Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame

Week	0 ppm		3,125 ppm			6,250 ppm			12,500 ppm		
	Feed (g)[a]	Body Weight (g)	Feed (g)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g)	Body Weight (g)	Dose (mg/kg)	Feed (g)	Body Weight (g)	Dose (mg/kg)
2	4.4	22.4	4.3	22.2	598	4.7	22.4	1,301	4.2	22.1	2,367
3	4.6	23.1	4.5	23.2	608	4.4	23.0	1,200	4.6	22.9	2,520
4	4.6	23.6	4.4	23.8	578	4.4	23.4	1,181	4.4	23.8	2,284
5	4.8	25.0	4.8	24.9	597	4.6	24.6	1,164	4.7	24.8	2,391
6	5.1	25.6	4.6	25.5	558	4.6	25.1	1,145	4.5	25.2	2,231
7	4.8	26.1	4.9	26.5	581	4.6	26.2	1,086	4.7	25.6	2,276
8	4.7	26.7	4.7	26.7	550	4.6	26.3	1,095	4.8	26.7	2,229
9	4.9	27.4	4.9	27.7	553	4.4	26.5	1,042	4.2	27.2	1,945
10	5.1	28.6	4.5	27.9	505	4.9	27.1	1,135	4.7	27.6	2,108
11	4.8	28.9	4.7	27.9	526	4.8	27.9	1,085	4.7	28.2	2,091
12	4.9	29.4	4.9	28.4	542	4.9	28.2	1,086	4.6	28.3	2,039
13	4.9	30.0	4.6	28.8	499	4.6	28.3	1,013	4.7	29.0	2,042
14	4.9	30.1	4.8	29.1	517	4.8	28.7	1,047	5.1	29.6	2,147
15	5.0	30.8	5.1	29.4	543	5.3	29.8	1,120	5.0	29.9	2,079
16	5.1	30.6	4.9	29.8	516	4.6	29.8	966	5.3	30.7	2,159
17	4.8	31.6	4.4	30.7	448	4.7	30.8	960	4.8	31.7	1,907
18	4.9	32.4	4.6	31.1	463	4.1	30.5	847	5.2	32.1	2,028
19	4.5	32.8	4.4	31.5	440	4.4	30.1	904	4.5	32.4	1,732
20	4.5	33.3	4.8	32.1	463	4.8	30.9	971	4.5	32.8	1,697
21	4.8	34.0	4.9	32.6	472	4.8	31.9	941	5.0	33.1	1,874
22	4.8	34.2	4.6	32.7	441	4.6	32.2	898	4.5	32.7	1,733
23	4.8	34.4	4.7	32.7	451	4.4	32.2	864	4.5	33.0	1,695
24	4.3	34.5	4.3	32.7	407	4.3	32.2	840	4.5	33.3	1,672
25	4.8	34.6	4.6	32.8	440	4.5	32.2	881	4.4	33.2	1,649
26	4.5	34.6	4.4	32.7	425	4.5	32.2	866	4.7	33.6	1,754
27	4.6	34.6	4.6	32.7	443	4.5	32.6	873	4.6	33.8	1,701
28	4.7	35.1	4.7	33.4	439	4.5	32.9	847	4.7	34.3	1,695
29	4.8	35.6	4.7	33.9	438	4.3	33.3	815	4.6	34.6	1,659
30	4.6	35.9	4.4	33.8	407	4.6	33.8	850	4.4	35.0	1,570
31	4.5	35.8	4.4	33.4	414	4.4	33.9	812	4.6	34.8	1,639
32	5.0	36.1	4.7	33.5	438	4.3	33.7	800	4.6	34.8	1,644
33	4.9	35.6	5.0	33.5	462	4.8	34.0	878	4.7	34.3	1,714
34	4.7	36.1	4.4	33.1	419	4.3	34.2	791	4.3	34.9	1,547
35	5.4	35.4	5.1	33.0	485	4.9	33.9	897	5.4	35.3	1,895
36	4.9	35.6	4.7	33.6	440	4.8	34.3	865	4.8	35.5	1,687
37	4.7	35.6	4.7	33.1	446	4.7	34.1	856	5.0	35.1	1,781
38	4.9	35.9	4.6	32.6	445	5.0	34.0	926	4.9	35.2	1,742
39	4.7	35.6	4.5	32.4	436	4.4	33.9	818	4.6	34.9	1,641
40	5.0	35.7	5.2	33.1	495	4.9	33.7	916	4.8	35.0	1,716
2-13	4.8	26.4	4.6	26.1	558	4.6	25.7	1,128	4.6	25.9	2,210
14-40	4.8	34.3	4.7	32.4	453	4.6	32.4	891	4.7	33.5	1,769

TABLE J5
Feed and Compound Consumption by Male Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame

Week	25,000 ppm			50,000 ppm		
	Feed (g)	Body Weight (g)	Dose (mg/kg)	Feed (g)	Body Weight (g)	Dose (mg/kg)
2	4.0	22.0	4,525	3.9	22.3	8,802
3	4.1	22.7	4,555	4.1	23.0	8,809
4	4.2	23.0	4,575	4.2	23.6	8,881
5	4.7	24.7	4,788	4.4	24.9	8,835
6	4.2	25.0	4,202	4.5	25.1	9,012
7	4.6	26.1	4,419	4.5	26.4	8,491
8	4.7	26.6	4,439	4.4	26.5	8,308
9	4.2	27.1	3,845	4.1	26.9	7,709
10	4.1	27.7	3,714	4.7	27.3	8,670
11	4.5	28.0	4,021	4.8	28.1	8,499
12	4.2	28.2	3,715	4.8	28.9	8,385
13	5.0	29.1	4,255	4.8	29.2	8,263
14	4.6	29.1	3,951	4.8	29.4	8,192
15	4.8	29.5	4,066	4.8	29.9	8,047
16	4.8	29.8	3,991	4.8	30.4	7,849
17	4.4	30.5	3,641	4.5	31.4	7,214
18	4.5	31.6	3,556	4.4	31.6	7,012
19	4.5	32.0	3,482	4.6	32.1	7,177
20	4.7	32.2	3,642	4.7	32.7	7,242
21	4.8	33.0	3,673	4.5	32.8	6,908
22	4.5	33.1	3,376	4.4	33.1	6,639
23	4.6	33.0	3,447	4.3	33.0	6,458
24	4.4	33.0	3,310	4.3	33.4	6,495
25	4.3	32.9	3,281	4.3	33.3	6,481
26	4.4	33.1	3,335	4.4	33.5	6,551
27	4.5	33.3	3,397	4.5	33.7	6,626
28	4.6	33.9	3,397	4.4	33.8	6,559
29	4.4	34.3	3,201	4.3	33.9	6,382
30	4.6	34.6	3,347	4.4	34.3	6,474
31	4.6	34.5	3,332	4.4	34.4	6,455
32	4.4	34.6	3,202	4.4	34.0	6,442
33	4.8	34.6	3,488	4.6	33.9	6,844
34	4.4	35.1	3,133	4.4	34.4	6,462
35	4.5	34.8	3,258	4.8	34.3	7,040
36	4.7	35.3	3,346	4.7	34.3	6,871
37	4.7	34.9	3,364	4.6	33.9	6,811
38	4.7	35.0	3,338	4.7	34.1	6,883
39	4.6	35.4	3,261	4.6	34.4	6,738
40	4.8	35.6	3,388	4.8	34.2	7,042
2-13	4.4	25.8	4,254	4.4	26.0	8,555
14-40	4.6	33.3	3,452	4.5	33.1	6,885

[a] Grams of feed consumed per animal per day
[b] Milligrams of aspartame consumed per kilogram body weight per day

TABLE J6
Feed and Compound Consumption by Female Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame

Week	0 ppm		3,125 ppm			6,250 ppm			12,500 ppm		
	Feed (g)[a]	Body Weight (g)	Feed (g)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g)	Body Weight (g)	Dose (mg/kg)	Feed (g)	Body Weight (g)	Dose (mg/kg)
2	4.8	18.7	5.4	19.0	887	4.5	18.4	1,517	4.3	18.6	2,920
3	4.8	19.8	4.9	20.3	758	4.9	19.8	1,541	4.4	20.0	2,756
4	4.9	20.7	4.8	21.0	712	4.5	20.6	1,351	4.4	20.8	2,659
5	5.1	21.2	5.3	21.6	769	5.3	21.9	1,511	4.8	21.4	2,796
6	5.1	22.1	4.9	22.3	684	4.8	22.4	1,346	5.1	22.3	2,884
7	5.0	22.5	5.5	23.0	753	4.7	22.6	1,298	5.0	22.8	2,716
8	5.5	21.8	5.7	22.3	801	5.4	22.6	1,508	5.5	22.9	2,981
9	5.7	22.7	5.0	23.1	672	4.5	23.0	1,227	4.7	23.0	2,559
10	5.9	23.1	5.0	23.4	666	5.4	22.8	1,479	4.8	23.7	2,551
11	5.9	23.0	5.4	23.6	716	4.8	23.7	1,258	5.1	23.9	2,673
12	5.6	23.3	5.2	24.1	675	4.8	24.0	1,236	5.1	24.7	2,581
13	4.8	23.5	4.5	24.5	573	4.4	23.8	1,166	4.5	25.0	2,237
14	5.3	23.3	5.9	24.2	762	5.5	24.0	1,431	5.9	24.6	2,989
15	6.3	24.3	5.9	25.0	743	6.2	25.1	1,540	6.0	25.1	2,985
16	5.7	24.3	5.8	24.9	729	5.5	25.1	1,363	6.1	25.3	2,994
17	5.2	24.4	4.7	25.7	571	4.7	26.1	1,128	4.7	25.8	2,269
18	5.1	24.8	4.7	25.8	569	4.4	26.1	1,055	4.8	25.8	2,349
19	4.2	25.1	4.5	26.3	540	4.2	26.1	1,002	4.5	26.2	2,132
20	4.6	25.3	4.8	26.7	565	5.0	25.5	1,229	4.7	26.3	2,208
21	5.2	25.7	5.4	27.2	619	5.1	26.1	1,225	5.1	26.9	2,376
22	4.8	25.4	4.9	27.0	570	4.6	26.7	1,071	4.5	26.8	2,089
23	5.1	25.3	4.8	26.8	556	4.8	26.7	1,123	4.9	26.6	2,286
24	4.7	25.7	4.5	27.2	516	4.5	26.9	1,034	4.5	26.5	2,097
25	4.6	25.9	4.6	27.4	527	4.6	26.4	1,081	4.5	26.7	2,125
26	4.9	26.2	4.6	27.3	528	4.6	26.7	1,077	4.6	26.8	2,138
27	4.7	25.6	4.6	26.9	531	4.7	26.5	1,115	4.8	27.0	2,208
28	4.4	26.2	4.5	27.9	501	4.6	27.4	1,045	4.5	27.4	2,042
29	4.6	26.8	4.6	28.1	509	4.3	27.0	986	4.5	27.6	2,049
30	4.4	26.4	4.4	28.5	480	4.3	27.2	993	4.2	27.8	1,905
31	4.4	25.6	4.4	28.2	486	4.6	28.3	1,006	4.3	27.7	1,950
32	4.5	26.2	4.5	28.1	497	4.5	27.8	1,019	4.2	27.7	1,897
33	5.8	26.8	5.1	28.0	568	4.8	28.1	1,078	4.7	27.5	2,135
34	4.9	27.1	4.3	28.4	479	4.4	28.3	973	4.4	27.7	1,968
35	5.8	27.0	5.9	28.0	656	5.3	27.9	1,177	5.5	28.0	2,439
36	5.4	27.0	5.2	28.1	575	4.9	28.1	1,093	5.1	28.0	2,260
37	5.2	26.8	5.1	27.6	573	5.0	27.7	1,122	5.1	27.8	2,284
38	5.0	26.9	4.9	27.7	550	4.7	28.0	1,061	4.8	27.8	2,155
39	4.8	26.7	4.5	27.6	507	4.7	28.3	1,036	4.6	28.2	2,054
40	5.4	27.4	5.4	27.9	600	5.5	28.8	1,195	5.2	28.6	2,296
2-13	5.2	21.9	5.1	22.4	722	4.8	22.2	1,370	4.8	22.4	2,693
14-40	5.0	25.9	4.9	27.1	567	4.8	26.9	1,121	4.8	27.0	2,247

TABLE J6
Feed and Compound Consumption by Female Cdkn2a Deficient Mice in the 9-Month Feed Study of Aspartame

Week	25,000 ppm			50,000 ppm		
	Feed (g)	Body Weight (g)	Dose (mg/kg)	Feed (g)	Body Weight (g)	Dose (mg/kg)
2	4.3	18.6	5,761	4.1	18.8	10,888
3	4.3	19.6	5,434	4.2	20.0	10,466
4	4.2	20.3	5,204	4.4	20.7	10,640
5	4.6	20.9	5,479	5.0	21.9	11,426
6	4.8	21.8	5,514	5.0	22.5	11,210
7	5.4	22.3	6,050	4.9	22.6	10,898
8	5.1	22.0	5,794	5.3	22.5	11,842
9	4.3	22.1	4,907	4.4	22.8	9,617
10	4.6	22.8	5,069	5.1	23.1	11,074
11	5.1	22.9	5,576	5.3	23.6	11,196
12	4.7	23.1	5,039	5.0	23.9	10,461
13	4.8	23.8	5,013	4.5	23.9	9,453
14	5.1	23.3	5,508	5.6	24.2	11,475
15	5.9	23.8	6,215	5.5	24.8	11,035
16	5.3	23.7	5,616	5.1	24.5	10,383
17	4.5	24.2	4,688	4.8	25.6	9,320
18	4.5	24.9	4,503	4.5	25.7	8,794
19	4.1	25.1	4,117	4.4	25.8	8,460
20	4.7	25.1	4,675	5.1	26.0	9,808
21	5.0	25.6	4,906	5.1	26.5	9,620
22	4.7	25.4	4,665	4.8	26.5	9,073
23	4.7	25.7	4,551	4.9	26.7	9,144
24	4.5	25.4	4,403	4.6	26.6	8,674
25	4.5	25.3	4,425	4.3	26.2	8,297
26	4.5	25.4	4,436	4.5	26.2	8,651
27	4.8	25.6	4,653	4.8	26.7	8,955
28	4.5	25.9	4,374	4.6	26.9	8,461
29	4.3	26.1	4,094	4.4	26.7	8,267
30	4.5	26.4	4,259	4.4	27.1	8,172
31	4.3	26.3	4,127	4.4	27.5	8,000
32	4.7	26.7	4,365	4.5	27.4	8,234
33	4.6	26.5	4,335	5.3	27.6	9,700
34	4.6	26.9	4,233	4.3	28.1	7,596
35	4.9	26.9	4,534	5.1	27.8	9,251
36	4.8	26.4	4,519	5.1	28.0	9,129
37	5.0	26.8	4,655	4.9	27.7	8,789
38	4.9	26.6	4,613	5.1	27.7	9,244
39	4.7	27.1	4,344	4.5	27.8	8,069
40	5.1	26.9	4,697	5.1	28.3	9,044
2-13	4.7	21.7	5,403	4.8	22.2	10,764
14-40	4.7	25.7	4,611	4.8	26.7	9,024

[a] Grams of feed consumed per animal per day
[b] Milligrams of aspartame consumed per kilogram body weight per day

National Toxicology Program
National Institute of Environmental Health Sciences
National Institutes of Health
P.O. Box 12233, MD K2-05
Durham, NC 27709
Tel: 984-287-3211
ntpwebrequest@niehs.nih.gov

https://ntp.niehs.nih.gov

ISSN 1556-5246