NTP TECHNICAL REPORT

ON THE

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

# IN FVB/N-TgN(v-*Ha-ras*)Led (Tg.AC) HEMIZYGOUS MICE

# AND CARCINOGENICITY STUDIES OF ASPARTAME

# IN B6.129-*Trp53*$^{tm1Brd}$ (N5) HAPLOINSUFFICIENT MICE

(FEED STUDIES)

Scheduled Peer Review Date: May 22, 2003

NOTICE

This is a DRAFT Technical Report prepared for public review and comment. Until this DRAFT has been reviewed and approved by the NTP Board of Scientific Counselors' Technical Reports Review Subcommittee in public session, the interpretations described herein do not represent the official scientific position of the National Toxicology Program. Following peer review, readers should contact the NTP for the final version of this Technical Report.

NTP GMM 1

NIH Publication No. 03-4459

National Toxicology Program

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

The studies described in this Technical Report series were designed and conducted to characterize the toxicologic potential, including carcinogenic activity, of selected agents in laboratory animals that have been genetically modified. These genetic modifications may involve inactivation of selected tumor suppressor functions or activation of oncogenes that are commonly observed in human cancers. This may result in a rapid onset of cancer in the genetically modified animal when exposure is to agents that act directly or indirectly on the affected pathway. An absence of a carcinogenic response may reflect either an absence of carcinogenic potential of the agent or that the selected model does not harbor the appropriate genetic modification to reduce tumor latency and allow detection of carcinogenic activity under the conditions of these subchronic studies. Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

Details about ongoing and completed NTP studies are available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov. Abstracts of all NTP Technical Reports and full versions of the most recent reports and other publications are available from the NIEHS' Environmental Health Perspectives (EHP) http://ehp.niehs.nih.gov (866-541-3841 or 919-653-2590). In addition, printed copies of these reports are available from EHP as supplies last.

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NIH Publication No. 03-4459

National Toxicology Program

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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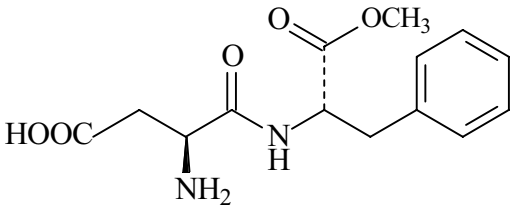
# ABSTRACT




**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 TgAC hemizygous strain and the p53 haploinsufficient strain, and an uncharacterized model, the Cdkn2a deficient strain. This report focuses on the findings in the Tg.AC hemizygous and p53 haploinsufficient mouse models. Male and female Tg.AC hemizygous and p53 haploinsufficient mice were exposed to aspartame (greater than 98% pure) in feed 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. There were no neoplasms or nonneoplastic lesions that were attributed to exposure to 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.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9.

## Summary of the 9-Month Carcinogenesis and Genetic Toxicology Studies of Aspartame

| | Male p53 Haploinsufficient Mice | Female p53 Haploinsufficient Mice |
|---|---|---|
| **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** | | |
| *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*: | Positive in p53 haploinsufficient females; negative in Tg.AC hemizygous males and females, p53 haploinsufficient males, and 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 Technical 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 Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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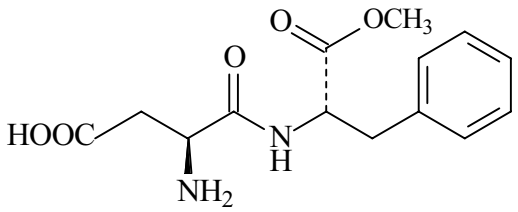
## SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

**NOTE:** A summary of the Technical Reports Review Subcommittee's remarks will appear in a future draft of this report.

# INTRODUCTION

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## 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 analog 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 average daily 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,

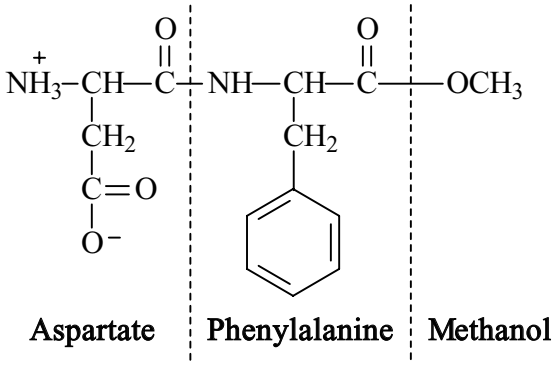



**FIGURE 1**
**Chemical Structure of Aspartame with its Components Indicated by the Dotted Lines** (Stegink, 1987)

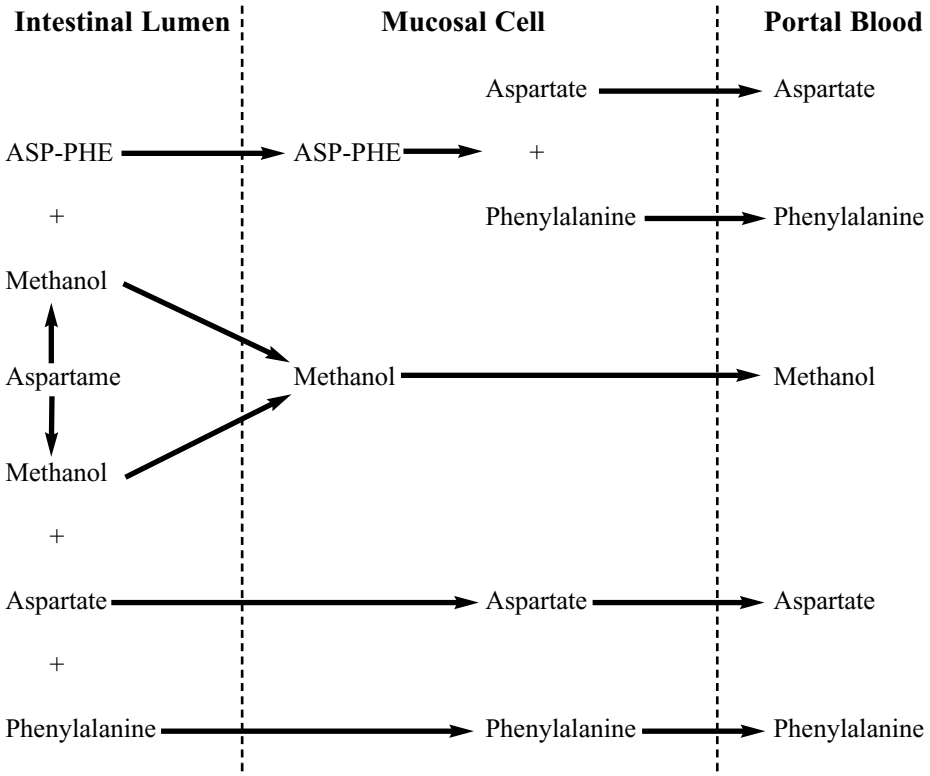



**FIGURE 2**
**Hydrolysis of Aspartame** (Stegink, 1987)

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 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 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 Den 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 at 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 occurance of gliomas at a relatively early age in some of the exposed groups. A total of seven

brain tumors occurred in exposed males, and a total of five occurred in females; one occurred in a control male. However, there were some brain tumor diagnosis inconsistencies among the various groups that reviewed these lesions.

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, 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 ependymonma 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 is little 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. Cutaneous papillomas at various sites have been reported at 10% and 7% incidence in 26-week-old control male and female Tg.AC mice, respectively (Mahler *et al.,*1998). Cutaneous papillomas occuring 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 an incidence of 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 Technical Report for the studies of aspartame and in a companion Technical Report for the studies of acesulfame potassium (NTP, 2003a) because this model has been reported to detect both nongenotoxic and genotoxic carcinogens (Spalding *et al.,* 1993; Tennant *et al.,* 1995*,* 1996; Pitchard, 2003).

### *B6.129-Trp53$^{tm1Brd}$ (N5) 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 is labile and exists at very low concentrations in normal cells; in DNA damaged cells or a variety of transformed cell lines, however, it is expressed in high amounts, and is believed to contribute to transformation and malignancy. p53 is a DNA-binding protein containing DNA-binding, oligomerization, and transcription activation domains. Many amino acid residues may be phosphorylated or acetylated, which may determine p53 function. 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 frequently occur in human cancers, and are unable to function as tumor suppressors. 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) 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 supressor 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. This report focuses primarily on the Tg.AC hemizygous and p53 haploinsufficient mouse models. Results with the Cdkn2a deficient mouse are reported in Appendix I and commented on briefly in the discussion.

# MATERIALS AND METHODS

## Procurement and Characterization of Aspartame

Aspartame was obtained from Spectrum Quality Products, Inc. (Gardena, CA) via Research Triangle Institute (RTI), (Research Triangle Park, NC), 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, RTI, and the study laboratory; 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 G1 and G2.

The purity of lot 8415-14-02 RTI was determined by the analytical chemistry laboratory using a high-performance liquid chromatography (HPLC) system.

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 System 2. No degradation of the bulk chemical was detected.

## Preparation and Analysis of Dose Formulations

The dose formulations were prepared every two weeks by mixing aspartame with feed (Table G1). Homogeneity and stability studies of dose formulations were performed by the analytical chemistry laboratory and the study laboratory with 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 G2). Animal room samples of these dose formulations were also analyzed, and 27 of 35 of the animal room samples were within 10% of the target concentrations (Table G2). 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 (Tg.AC heterozygous) and B6.129-*Trp53*$^{tm1Brd}$ (N5) haploinsufficient (p53 haploinsufficient) mice were obtained from Taconic Farms (Germantown, NY) for use in the 9-month studies. Tg.AC heterozygous mice were quarantined for 11 days and p53 haploinsufficient mice were quarantined for 14 days before the beginning of the studies. Five male and five female mice in the control and 50,000 ppm groups were randomly selected for parasite evaluation and gross observation of disease. Tg.AC heterozygous mice were approximately 6 weeks old and p53 haploinsufficient mice were approximately 7 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program; 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 comtamination. 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 all 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 and p53 haploinsufficient 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 that 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 |
|---|---|
| **Study Laboratory**<br>BioReliance (Rockville, MD) | BioReliance (Rockville, MD) |
| **Strain**<br>FVB/N-TgN(v-Ha-*ras*)Led (Tg.Ac) hemizygous | B6.129-*Trp53*$^{tm1Brd}$ (N5) haploinsufficient |
| **Animal Source**<br>Taconic Farms, Inc. (Germantown, NY) | Taconic Farms, Inc. (Germantown, NY) |
| **Time Held Before Studies**<br>11 days | 14 days |
| **Average Age When Studies Began**<br>6 weeks | 7 weeks |
| **Date of First Exposure**<br>October 11, 1999 | October 14, 1999 |
| **Duration of Exposure**<br>39 weeks | 40 weeks |
| **Date of Last Exposure**<br>July 11, 2000 | July 17, 2000 |
| **Necropsy Dates**<br>July 10-11, 2000 | July 13-17, 2000 |
| **Average Age at Necropsy**<br>45 weeks | 46 weeks |
| **Size of Study Groups**<br>15 males and 15 females | 15 males and 15 females |
| **Method of Distribution**<br>Animals were distributed randomly into groups of approximately equal initial mean body weights. | Same as Tg.AC heterozygous mice |
| **Animals per Cage**<br>1 | 1 |
| **Method of Animal Identification**<br>Tail tattoo | Tail tattoo |
| **Diet**<br>Irradiated NTP-2000 open formula meal (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum* | Same as Tg.AC heterozygous mice |

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

| Tg.AC Hemizygous Mice | p53 Haploinsufficient 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 |
| **Cages** | |
| Polycarbonate cages (Lab Products, Inc., Seaford, DE), changed weekly | 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 |
| **Cage Filters** | |
| Remay 2016 (Snow Filtration, West Chester, OH), changed every 2 weeks | 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 |
| **Animal Room Environment** | |
| Temperature: 72° ± 3° F<br>Relative humidity: 50% ± 15%<br>Room fluorescent light: 12 hours/day<br>Room/Chamber air changes: 10/hour | Temperature: 72° ± 3° F<br>Relative humidity: 50% ± 15%<br>Room fluorescent light: 12 hours/day<br>Room/Chamber 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* |
| **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. |
| **Method of Sacrifice** | |
| 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 |

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

| Tg.AC Hemizygous Mice | p53 Haploinsufficient 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, brain, bone with marrow, clitoral gland, esophagus, eye, gallbladder, Harderian gland, heart with aorta, kidney, large intestine (cecum, colon, rectum), small intestine (dudenum, jejunum, ilieum), larynx, liver, lung and mainstem bronchi, mammary gland (with mesenteric lymph nodes), nose, oral cavity, ovary, pancreas, parathyroid gland, pharynx, 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, brain, bone with marrow, clitoral gland, esophagus, eye, gallbladder, Harderian gland, heart with aorta, kidney, large intestine (cecum, colon, rectum), small intestine (dudenum, jejunum, ilieum), larynx, liver, lung and mainstem bronchi, mammary gland (with mesenteric lymph nodes), nose, oral cavity, ovary, pancreas, parathyroid gland, pharynx, 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. |
| 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, preputial gland, prostate gland, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), 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) and is presented in the form of graphs. Animals found dead of other than natural causes were censored from the survival analyses; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

### Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A4, B1, B4, C1, C4, D1, and D4 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, and D3) 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, and D3 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 Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

## Genetic Toxicology

The genetic toxicity of 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 E.

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 papillomas each by week 16 (data not shown). This is consistent with historical rates found in other studies (Tennent *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**.

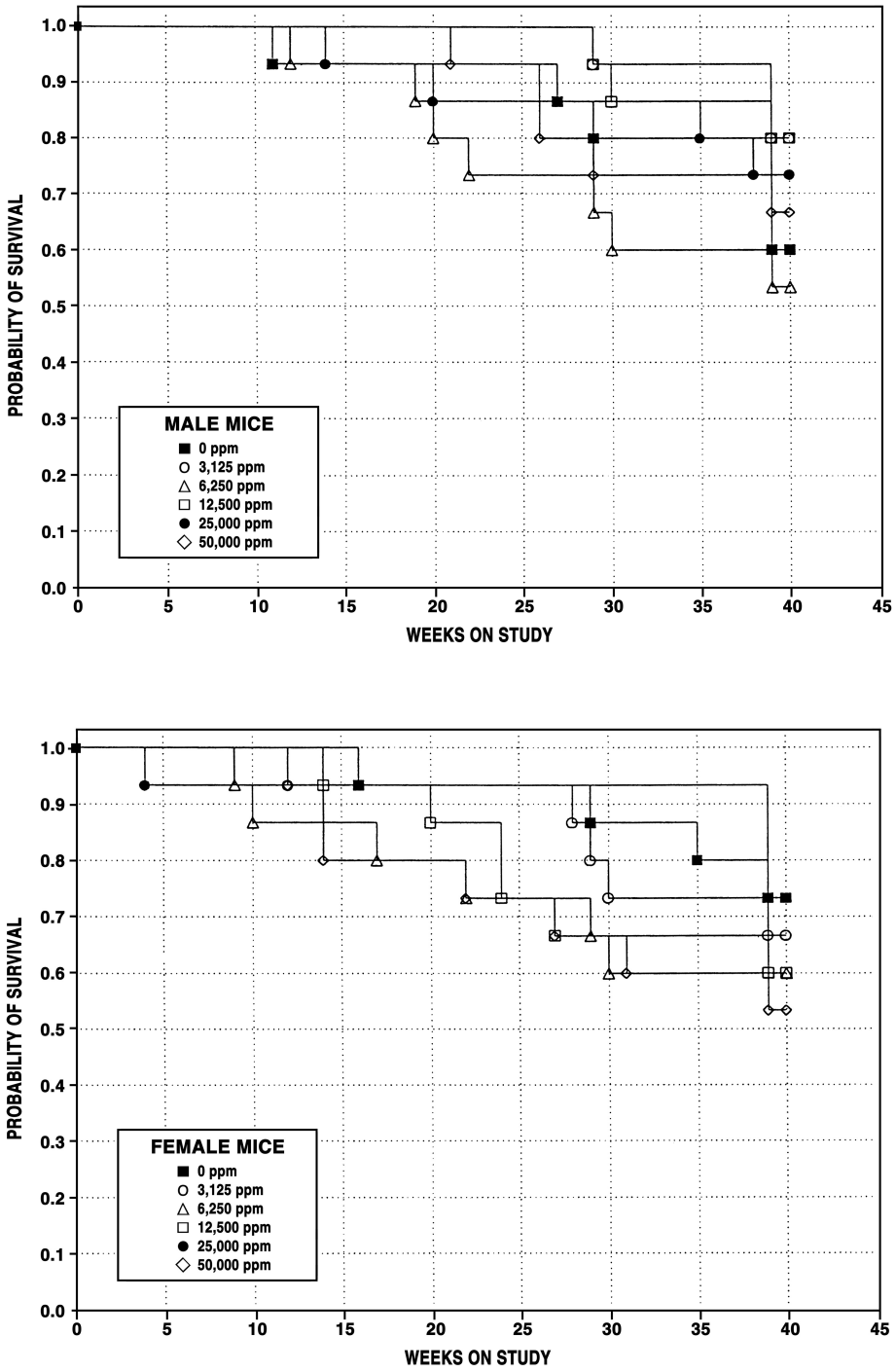


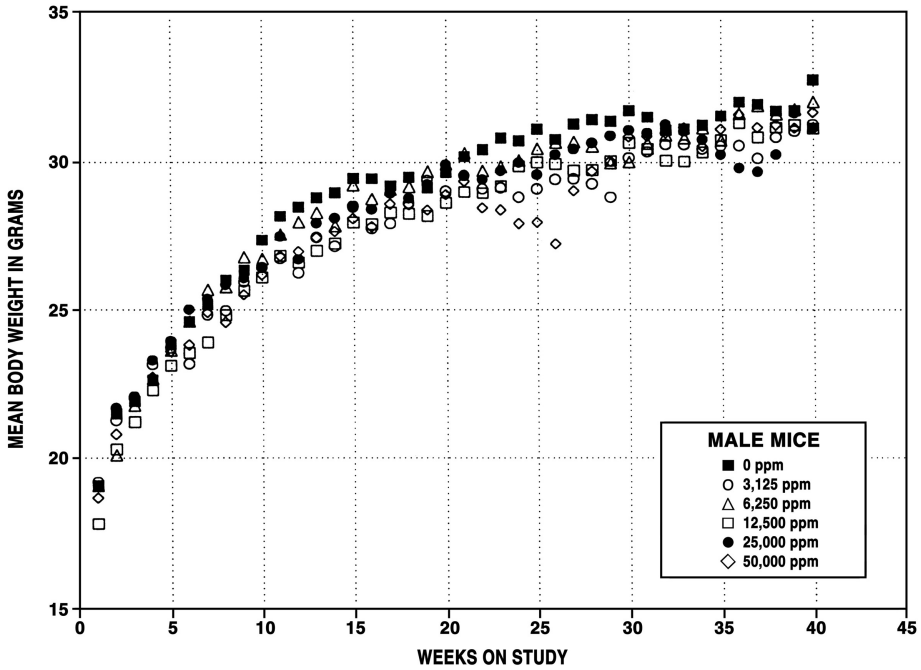

**FIGURE 3**
**Kaplan-Meier Survival Curves for Male and Female Tg.AC Hemizygous Mice Exposed to Aspartame in Feed for 9 Months**

### *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 (Figure 4; Tables 3 and 4). Feed consumption by the exposed groups was similar to that by the control groups (Tables H1 and H2). 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 apartame 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 F1).



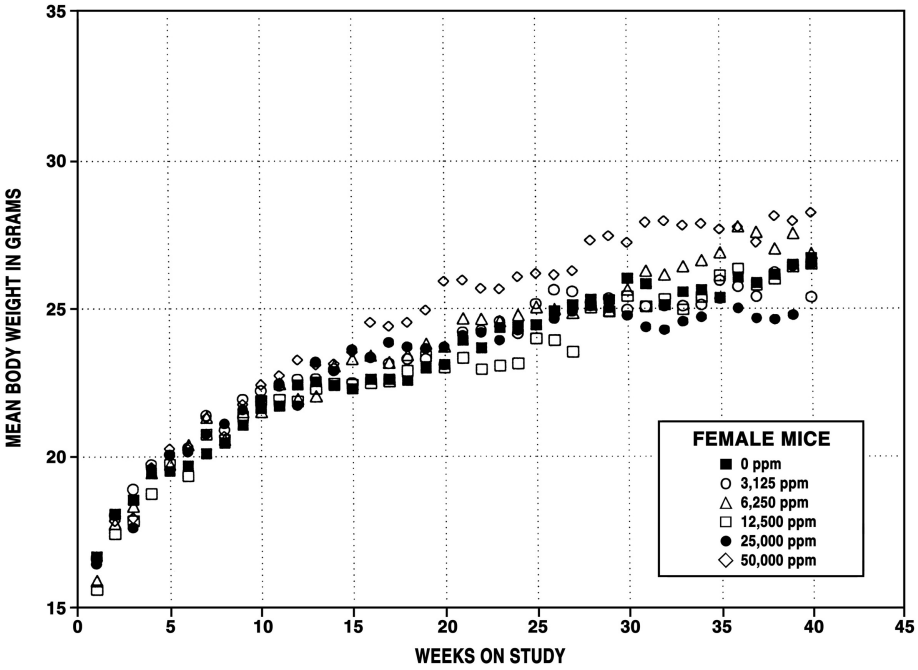




**FIGURE 4**
**Growth Curves for Male and Female Tg.AC Hemizygous Mice Exposed to Aspartame in Feed for 9 Months**

**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 | |

### *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:* The incidences of alveolar/bronchiolar adenoma in 6,250, 12,500, 25,000, and 50,000 ppm males were slightly increased (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:* The incidences of carcinoma were slightly increased in 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 incidences of germ cell degeneration of the testis were slightly increased in all exposed groups of males (Tables 5 and A4). The lesion was predominanetly 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 low incidence of this lesion indicates the finding is sporadic, strain specific, and not related 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

**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

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.

## 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.

**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 |

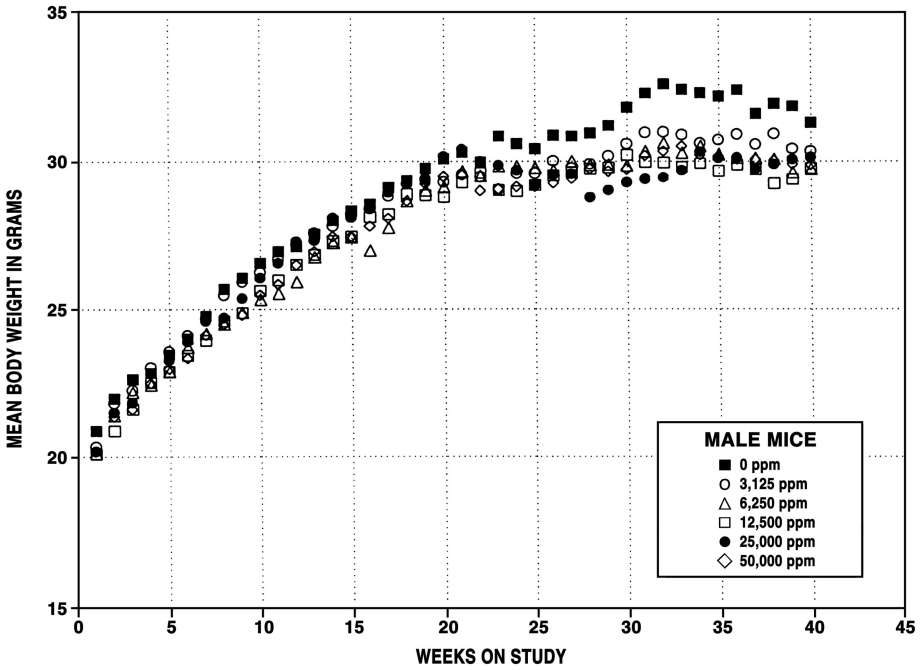
[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice).
[c] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed group columns.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

### *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; however, the differences were likely related to variability of body weights within the exposed groups (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 H3 and H4). 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 F2).



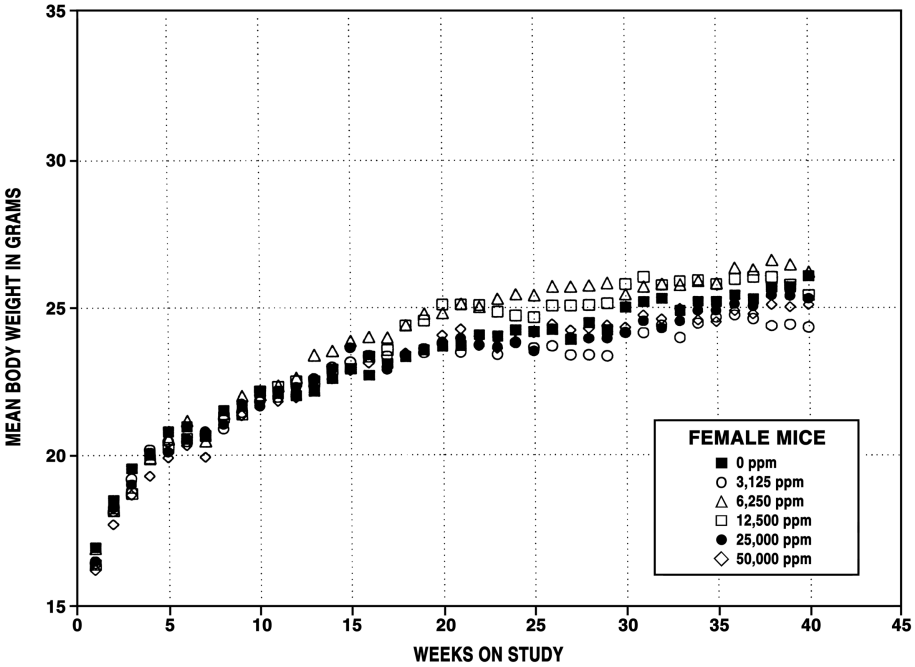




**FIGURE 5**
**Growth Curves for Male and Female p53 Haploinsufficient Mice Exposed to Aspartame in Feed for 9 Months**

**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 | | | 50,000 ppm | | |
|---|---|---|---|---|---|---|
| | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | 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 | | 23.3 | 95 | |

### *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.

## 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 E1). 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.

An acute bone marrow micronucleus test was conducted with aspartame administered by gavage to male F344/N rats. The percent of polychromatic erthyrocytes was not greatly altered in treated rats (Table E2). However, a slight decrease in percent polychromatic erthyrocytes occurred in the 1,000 mg/kg group, and in the positive controls the percent of polychromatic erthyrocytes 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 E3 and E5). Negative results were also obtained in male p53 haploinsufficient mice (Table E4); 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.

# 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 protocols in place 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 (Appendix I), 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 been reported to historically occur 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 papillomas of the skin or forestomach 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 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 either 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, 2003b) 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 there were no treatment-related neoplasms or nonneoplastic lesions 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 run 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 (Appendix I). 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 administered aspartame in feed at concentrations up to 50,000 ppm for 9 months. There was also no evidence of enhanced tumor formation in a study of a similar design in a p16 tumor suppressor mouse model; 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.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9.

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# 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-2
**TABLE A2** Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame . . . . . A-4
**TABLE A3** Statistical Analysis of Primary Neoplasms in Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame . . . . . A-16
**TABLE A4** Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame . . . . . A-18

**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** | 076 | 183 | 197 | 271 | 271 | 271 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | |
| **Carcass ID Number** | 004 | 014 | 015 | 006 | 012 | 013 | 001 | 002 | 003 | 005 | 007 | 008 | 009 | 010 | 011 | 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** | 076 | 183 | 197 | 271 | 271 | 271 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | |
| **Carcass ID Number** | 004 | 014 | 015 | 006 | 012 | 013 | 001 | 002 | 003 | 005 | 007 | 008 | 009 | 010 | 011 | 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** | 198 | 271 | 271 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | |
| **Carcass ID Number** | 029 | 024 | 025 | 016 | 017 | 018 | 019 | 020 | 021 | 022 | 023 | 026 | 027 | 028 | 030 | 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** | 198 | 271 | 271 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | |
| **Carcass ID Number** | 029 | 024 | 025 | 016 | 017 | 018 | 019 | 020 | 021 | 022 | 023 | 026 | 027 | 028 | 030 | 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** | 078 | 132 | 138 | 150 | 197 | 207 | 271 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | |
| **Carcass ID Number** | 039 | 033 | 043 | 037 | 034 | 042 | 031 | 032 | 035 | 036 | 038 | 040 | 041 | 044 | 045 | 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** | 078 | 132 | 138 | 150 | 197 | 207 | 271 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | |
| **Carcass ID Number** | 039 | 033 | 043 | 037 | 034 | 042 | 031 | 032 | 035 | 036 | 038 | 040 | 041 | 044 | 045 | 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** | 199 | 206 | 271 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | |
| **Carcass ID Number** | 058 | 050 | 046 | 047 | 048 | 049 | 051 | 052 | 053 | 054 | 055 | 056 | 057 | 059 | 060 | 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 | |
| **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** | 199 | 206 | 271 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | |
| **Carcass ID Number** | 058 | 050 | 046 | 047 | 048 | 049 | 051 | 052 | 053 | 054 | 055 | 056 | 057 | 059 | 060 | 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** | 096 | 138 | 239 | 261 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | |
| **Carcass ID Number** | 061 | 075 | 068 | 072 | 062 | 063 | 064 | 065 | 066 | 067 | 069 | 070 | 071 | 073 | 074 | 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** | 096 | 138 | 239 | 261 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | |
| **Carcass ID Number** | 061 | 075 | 068 | 072 | 062 | 063 | 064 | 065 | 066 | 067 | 069 | 070 | 071 | 073 | 074 | 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** | 141 | 180 | 180 | 200 | 271 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | |
| **Carcass ID Number** | 089 | 084 | 090 | 080 | 083 | 076 | 077 | 078 | 079 | 081 | 082 | 085 | 086 | 087 | 088 | 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** | 141 | 180 | 180 | 200 | 271 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | |
| **Carcass ID Number** | 089 | 084 | 090 | 080 | 083 | 076 | 077 | 078 | 079 | 081 | 082 | 085 | 086 | 087 | 088 | 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 | |
| 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 . . . . . B-2
**TABLE B2** Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame . . . . . B-4
**TABLE B3** Statistical Analysis of Primary Neoplasms in Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame . . . . . B-16
**TABLE B4** Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame . . . . . B-18

**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** | 109 | 200 | 242 | 270 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | |
| **Carcass ID Number** | 117 | 111 | 106 | 104 | 107 | 108 | 109 | 110 | 112 | 113 | 115 | 116 | 118 | 119 | 120 | 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** | 109 | 200 | 242 | 270 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | |
| **Carcass ID Number** | 117 | 111 | 106 | 104 | 107 | 108 | 109 | 110 | 112 | 113 | 115 | 116 | 118 | 119 | 120 | 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** | 081 | 192 | 198 | 206 | 271 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | |
| **Carcass ID Number** | 132 | 121 | 134 | 133 | 130 | 122 | 123 | 124 | 125 | 126 | 127 | 128 | 129 | 131 | 135 | 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** | 081 | 192 | 198 | 206 | 271 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | |
| **Carcass ID Number** | 132 | 121 | 134 | 133 | 130 | 122 | 122 | 122 | 122 | 122 | 122 | 122 | 122 | 131 | 135 | 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** | 061 | 067 | 113 | 148 | 200 | 207 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | |
| **Carcass ID Number** | 138 | 148 | 141 | 139 | 137 | 149 | 136 | 140 | 142 | 143 | 144 | 145 | 146 | 147 | 150 | 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** | 061 | 067 | 113 | 148 | 200 | 207 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | |
| **Carcass ID Number** | 138 | 148 | 141 | 139 | 137 | 149 | 136 | 140 | 142 | 143 | 144 | 145 | 146 | 147 | 150 | 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** | 097 | 134 | 165 | 165 | 184 | 271 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | |
| **Carcass ID Number** | 156 | 153 | 154 | 160 | 164 | 159 | 151 | 152 | 155 | 157 | 158 | 161 | 162 | 163 | 165 | 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** | 097 | 134 | 165 | 165 | 184 | 271 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | |
| **Carcass ID Number** | 156 | 153 | 154 | 160 | 164 | 159 | 151 | 152 | 155 | 157 | 158 | 161 | 162 | 163 | 165 | 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** | 023 | 267 | 269 | 270 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | |
| **Carcass ID Number** | 178 | 167 | 173 | 168 | 166 | 169 | 170 | 171 | 172 | 174 | 175 | 176 | 177 | 179 | 180 | 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** | 023 | 267 | 269 | 270 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | |
| **Carcass ID Number** | 178 | 167 | 173 | 168 | 166 | 169 | 170 | 171 | 172 | 174 | 175 | 176 | 177 | 179 | 180 | 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** | 079 | 092 | 092 | 151 | 184 | 215 | 269 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | |
| **Carcass ID Number** | 188 | 182 | 192 | 191 | 190 | 194 | 187 | 181 | 183 | 184 | 185 | 186 | 189 | 193 | 195 | 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** | 079 | 092 | 092 | 151 | 184 | 215 | 269 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | |
| **Carcass ID Number** | 188 | 182 | 192 | 191 | 190 | 194 | 187 | 181 | 183 | 184 | 185 | 186 | 189 | 193 | 195 | 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 . . . . . C-2

**TABLE C2** Individual Animal Tumor Pathology of Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame . . . . . C-4

**TABLE C3** Statistical Analysis of Primary Neoplasms in Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame . . . . . C-16

**TABLE C4** Summary of the Incidence of Nonneoplastic Lesions in Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame . . . . . C-17

**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 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) | (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** | 102 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 275 | 275 | |
| **Carcass ID Number** | 220 | 211 | 213 | 214 | 215 | 216 | 217 | 219 | 221 | 222 | 223 | 224 | 225 | 212 | 218 | 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** | 102 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 275 | 275 |
| **Carcass ID Number** | 220 | 211 | 113 | 114 | 115 | 116 | 117 | 119 | 221 | 222 | 223 | 224 | 225 | 112 | 118 |
| **Hematopoietic System** | | | | | | | | | | | | | | | |
| Bone marrow | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Lymph node, mandibular | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Lymph node, mesenteric | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Lymph node, mediastinal | A | + | M | + | + | + | + | + | + | + | + | + | + | + | + |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Thymus | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **Integumentary System** | | | | | | | | | | | | | | | |
| Mammary gland | M | M | M | M | M | M | M | M | M | M | M | M | M | M | M |
| Skin | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **Musculoskeletal System** | | | | | | | | | | | | | | | |
| Bone | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Skeletal muscle | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **Nervous System** | | | | | | | | | | | | | | | |
| Brain | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Peripheral nerve | | | | | | | | | | | + | | | | |
| Spinal cord | | | | | | | | | | | + | | | | |
| **Respiratory System** | | | | | | | | | | | | | | | |
| Larynx | M | M | M | M | M | M | M | M | + | M | M | + | + | + | M |
| Lung | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Nose | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Trachea | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **Special Senses System** | | | | | | | | | | | | | | | |
| Eye | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Harderian gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Zymbal's gland | + | + | + | + | + | + | + | + | M | + | M | M | + | + | + |
| **Urinary System** | | | | | | | | | | | | | | | |
| Kidney | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Urinary bladder | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **Systemic Lesions** | | | | | | | | | | | | | | | |
| Multiple organs | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

| | Total Tissues/ Tumors |
|---|---|
| **Hematopoietic System** | |
| Bone marrow | 14 |
| Lymph node, mandibular | 14 |
| Lymph node, mesenteric | 14 |
| Lymph node, mediastinal | 13 |
| Spleen | 15 |
| Thymus | 14 |
| **Integumentary System** | |
| Mammary gland | |
| Skin | 15 |
| **Musculoskeletal System** | |
| Bone | 15 |
| Skeletal muscle | 15 |
| **Nervous System** | |
| Brain | 14 |
| Peripheral nerve | 1 |
| Spinal cord | 1 |
| **Respiratory System** | |
| Larynx | 4 |
| Lung | 15 |
| Nose | 14 |
| Trachea | 14 |
| **Special Senses System** | |
| Eye | 14 |
| Harderian gland | 15 |
| Zymbal's gland | 12 |
| **Urinary System** | |
| Kidney | 14 |
| Urinary bladder | 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** | 227 | 228 | 230 | 232 | 233 | 236 | 239 | 240 | 226 | 229 | 231 | 234 | 235 | 237 | 238 | 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** | 114 | 210 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 275 | 275 | 275 | 275 | |
| **Carcass ID Number** | 246 | 249 | 241 | 242 | 243 | 245 | 248 | 251 | 252 | 254 | 255 | 244 | 247 | 250 | 253 | 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 | 275 | 275 | 275 | 275 | |
| **Carcass ID Number** | 257 | 258 | 259 | 260 | 261 | 262 | 264 | 265 | 266 | 268 | 270 | 256 | 263 | 267 | 269 | 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 | |
| **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 | 275 | 275 | 275 | 275 | |
| **Carcass ID Number** | 257 | 258 | 259 | 260 | 261 | 262 | 264 | 265 | 266 | 268 | 270 | 256 | 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** | 049 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 275 | 275 | 275 | 275 | 275 | |
| **Carcass ID Number** | 275 | 271 | 272 | 273 | 274 | 276 | 278 | 279 | 280 | 282 | 277 | 281 | 283 | 284 | 285 | 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** | 049 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 275 | 275 | 275 | 275 | 275 | |
| **Carcass ID Number** | 275 | 271 | 272 | 273 | 274 | 276 | 278 | 279 | 280 | 282 | 277 | 281 | 283 | 284 | 285 | 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** | 071 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 275 | 275 | 275 | |
| **Carcass ID Number** | 291 | 286 | 287 | 289 | 292 | 294 | 295 | 296 | 297 | 298 | 299 | 300 | 288 | 290 | 293 | 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** | 071 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 274 | 275 | 275 | 275 | |
| **Carcass ID Number** | 291 | 286 | 287 | 289 | 292 | 294 | 295 | 296 | 297 | 298 | 299 | 300 | 288 | 290 | 293 | 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] | — | 114 | — | — | — |
| Poly-3 test[d] | P=0.378N | —[f] | 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 . . . . . D-2

**TABLE D2** Individual Animal Tumor Pathology of Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame . . . . . D-4

**TABLE D3** Statistical Analysis of Primary Neoplasms in Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame . . . . . D-16

**TABLE D4** Summary of the Incidence of Nonneoplastic Lesions in Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame . . . . . D-17

**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** | 036 | 275 | 275 | 275 | 275 | 275 | 278 | 278 | 278 | 278 | 278 | 278 | 278 | 278 | 278 | |
| **Carcass ID Number** | 313 | 304 | 307 | 308 | 310 | 311 | 301 | 302 | 303 | 305 | 306 | 309 | 312 | 314 | 315 | 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** | 036 | 275 | 275 | 275 | 275 | 275 | 278 | 278 | 278 | 278 | 278 | 278 | 278 | 278 | 278 | |
| **Carcass ID Number** | 313 | 304 | 307 | 308 | 310 | 311 | 301 | 302 | 303 | 305 | 306 | 309 | 312 | 314 | 315 | 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** | 098 | 275 | 275 | 275 | 278 | 278 | 278 | 278 | 278 | 278 | 278 | 278 | 278 | 278 | 278 | |
| **Carcass ID Number** | 325 | 317 | 319 | 326 | 316 | 318 | 320 | 321 | 322 | 323 | 324 | 327 | 328 | 329 | 330 | 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** | 098 | 275 | 275 | 275 | 278 | 278 | 278 | 278 | 278 | 278 | 278 | 278 | 278 | 278 | 278 | |
| **Carcass ID Number** | 325 | 317 | 319 | 326 | 316 | 318 | 320 | 321 | 322 | 323 | 324 | 327 | 328 | 329 | 330 | Total Tissues/ 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** | 168 | 275 | 275 | 275 | 275 | 275 | 275 | 278 | 278 | 278 | 278 | 278 | 278 | 278 | 278 | |
| **Carcass ID Number** | 341 | 332 | 335 | 336 | 339 | 340 | 343 | 331 | 333 | 334 | 337 | 338 | 342 | 344 | 345 | 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** | 168 | 275 | 275 | 275 | 275 | 275 | 275 | 278 | 278 | 278 | 278 | 278 | 278 | 278 | 278 | |
| **Carcass ID Number** | 341 | 332 | 335 | 336 | 339 | 340 | 343 | 331 | 333 | 334 | 337 | 338 | 342 | 344 | 345 | 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** | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 275 | 278 | 278 | 278 | 278 | 278 | 278 | |
| **Carcass ID Number** | 346 | 349 | 350 | 353 | 354 | 355 | 357 | 359 | 360 | 347 | 348 | 351 | 352 | 356 | 358 | 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 | |
| **Carcass ID Number** | 346 | 349 | 350 | 353 | 354 | 355 | 357 | 359 | 360 | 347 | 348 | 351 | 352 | 356 | 358 | 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** | 275 | 275 | 275 | 275 | 275 | 275 | 278 | 278 | 278 | 278 | 278 | 278 | 278 | 278 | 278 | |
| **Carcass ID Number** | 361 | 364 | 367 | 369 | 374 | 375 | 362 | 363 | 365 | 366 | 368 | 370 | 371 | 372 | 373 | Total Tissues/ Tumors |
| **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** | 361 | 364 | 367 | 369 | 374 | 375 | 362 | 363 | 365 | 366 | 368 | 370 | 371 | 372 | 373 | 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** | 376 | 378 | 383 | 384 | 385 | 388 | 387 | 379 | 380 | 381 | 382 | 386 | 387 | 389 | 390 | 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** | 376 | 378 | 383 | 384 | 385 | 388 | 377 | 379 | 380 | 381 | 382 | 386 | 387 | 389 | 390 | 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
# GENETIC TOXICOLOGY

*SALMONELLA TYPHIMURIUM* MUTAGENICITY TEST PROTOCOL . . . . . E-2
RAT BONE MARROW MICRONUCLEUS TEST PROTOCOL . . . . . E-2
MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL . . . . . E-3
EVALUATION PROTOCOL . . . . . E-3
RESULTS . . . . . E-3
TABLE E1 Mutagenicity of Aspartame in *Salmonella typhimurium* . . . . . E-4
TABLE E2 Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Rats Treated with Aspartame by Gavage . . . . . E-6
TABLE E3 Frequency of Micronuclei in Peripheral Blood Erythrocytes of Tg.AC Hemizygous Mice Following Administration of Aspartame in Feed for 9 Months . . . . . E-7
TABLE E4 Frequency of Micronuclei in Peripheral Blood Erythrocytes of p53 Haploinsufficient Mice Following Administration of Aspartame in Feed for 9 Months . . . . . E-8
TABLE E5 Frequency of Micronuclei in Peripheral Blood Erythrocytes of Cdkn2a Deficient Mice Following Administration of Aspartame in Feed for 9 Months . . . . . E-9

# 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]. Solvent 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 Technical 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 E1). 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 E2). The percent of PCEs was not greatly altered in treated rats (Table E2). 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 E3 and E5). Negative results were also obtained in male p53 haploinsufficient mice (Table E4); 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 E1**
**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** (continued) | 0 | 115 ± 8.5 | 97 ± 5.2 | 95 ± 7.5 | 80 ± 5.0 |
| | 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** (continued) | 0 | 13 ± 2.8 | 9 ± 1.8 | 12 ± 1.2 |
| | 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 E1**
**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 E2**
**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*. (1990); PCE=polychromatic erythrocyte.
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control. Dosed group values are significant at P≤0.008.
[d] Solvent 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 E3**
**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.
[b] Mean ± standard error
[c] Pairwise comparison with the controls, 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 E4**
**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.
[b] Mean ± standard error
[c] Pairwise comparison with the controls, 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 E5**
**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.
[b] Mean ± standard error
[c] Pairwise comparison with the controls, 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 F
# ORGAN WEIGHTS
# AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

**TABLE F1** **Organ Weights and Organ-Weight-to-Body-Weight Ratios for Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame** . . . . . **F-2**
**TABLE F2** **Organ Weights and Organ-Weight-to-Body-Weight Ratios for p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame** . . . . . **F-4**

**Table F1**
**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 | 47.308 ± 1.065 | 43.784 ± 0.897 | 43.891 ± 1.083 | 44.124 ± 0.631 | 45.555 ± 1.409 | 47.419 ± 1.789 |
| 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 F1**
**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 | 48.231 ± 1.339 | 46.251 ± 0.992 | 46.090 ± 1.192 | 46.900 ± 1.741[c] | 45.380 ± 1.257 | 49.431 ± 2.673 |
| 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 F2**
**Organ Weights and Organ-Weight-to-Body-Weight Ratios for 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 | 25,000 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 | 43.762 ± 0.770 | 44.883 ± 0.734 | 44.879 ± 0.784 | 45.593 ± 0.876 | 45.765 ± 0.856 | 46.439 ± 1.409 |
| 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 F2**
**Organ Weights and Organ-Weight-to-Body-Weight Ratios for 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 | 25,000 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 | 48.244 ± 1.109[b] | 48.061 ± 1.005 | 45.994 ± 1.115 | 45.987 ± 1.037 | 46.918 ± 0.945 | 45.960 ± 0.919 |
| 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

# APPENDIX G
# CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES


Procurement and Characterization of Aspartame . . . . . G-2
Preparation and Analysis of Dose Formulations . . . . . G-2
Figure G1 Infrared Absorption Spectrum of Aspartame . . . . . G-4
Figure G2 Proton Nuclear Magnetic Resonance Spectrum of Aspartame . . . . . G-5
Table G1 Preparation and Storage of Dose Formulations in the 9-Month Feed Studies of Aspartame . . . . . G-6
Table G2 Results of Analyses of Dose Formulations Administered to Tg.AC Hemizygous Mice, p53 Haploinsufficient Mice, and Cdkn2a Deficient Mice in the 9-Month Studies of Aspartame . . . . . G-7

# CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

## Procurement and Characterization of Aspartame

Aspartame was obtained from Spectrum Quality Products, Inc. (Gardena, CA) via Research Triangle Institute (RTI), (Research Triangle Park, NC), 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, RTI, and the study laboratory; 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 G1 and G2.

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 two weeks by mixing aspartame with feed (Table G1). 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 with 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 G2). Animal room samples of these dose formulations were also analyzed, and 27 of 35 of the animal room samples were within 10% of the target concentrations (Table G2). Dose formulations were stored refrigerated, protected from light, in doubled polyethylene bags for up to 28 days.

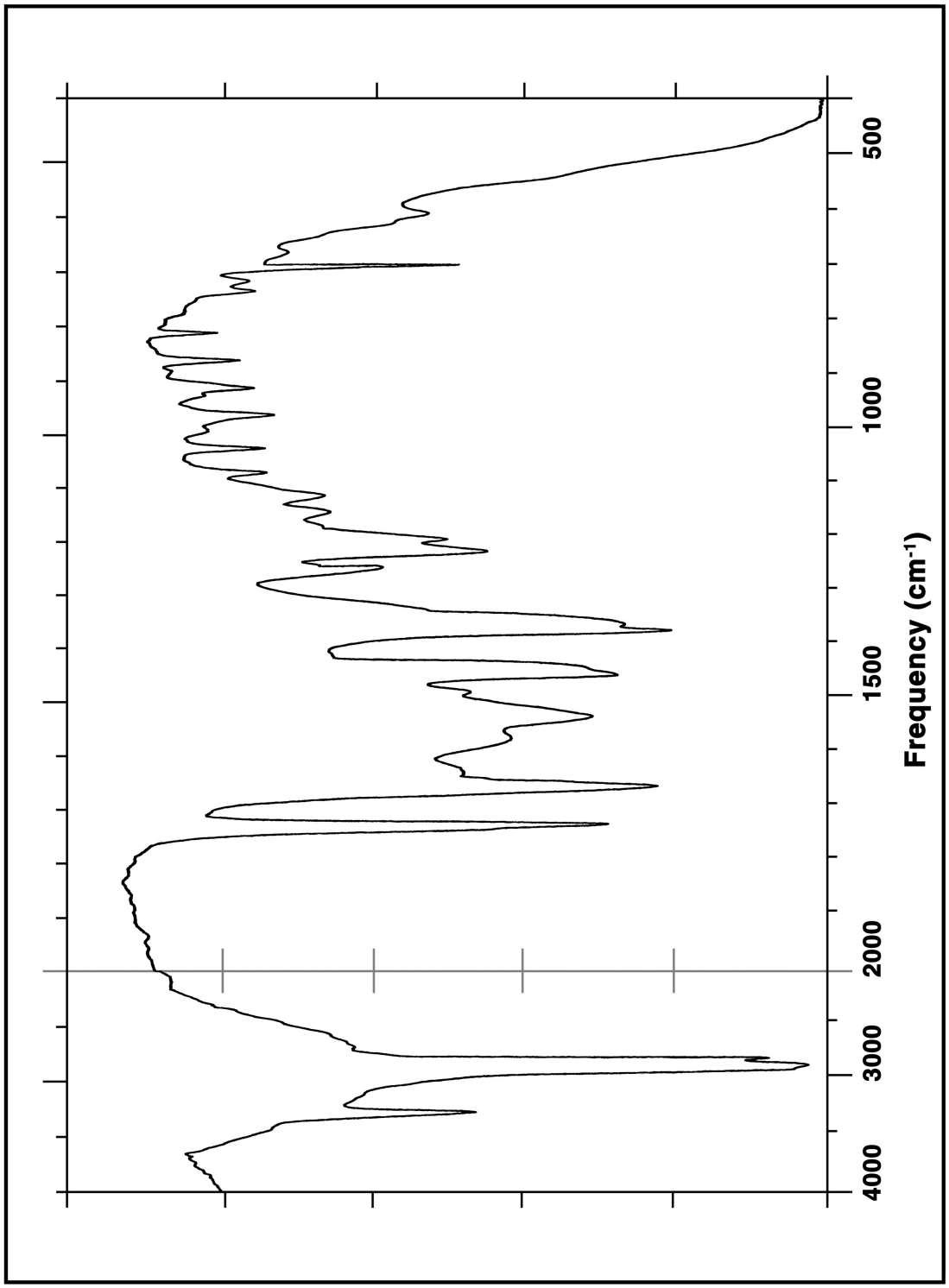


**FIGURE G1**
**Infrared Absorption Spectrum of Aspartame**

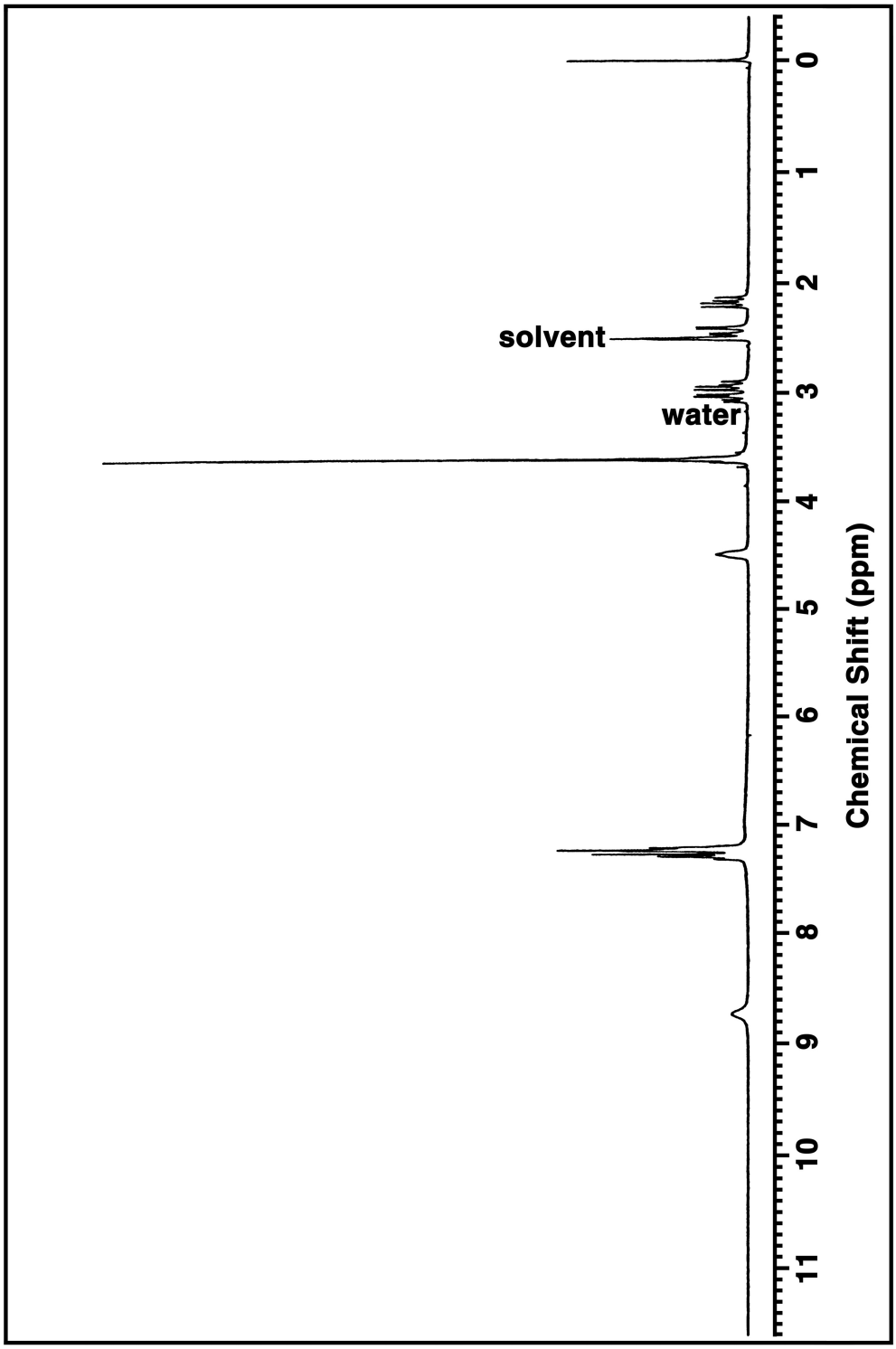


FIGURE G2
Proton Nuclear Magnetic Resonance Spectrum of Aspartame

**Table G1**
**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 two 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 (Rockville, MD)

**TABLE G2**
**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 G2**
**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 H
# FEED AND COMPOUND CONSUMPTION IN THE 9-MONTH FEED STUDIES OF ASPARTAME

**TABLE H1** Feed and Compound Consumption by Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame . . . . . H-2
**TABLE H2** Feed and Compound Consumption by Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame . . . . . H-4
**TABLE H3** Feed and Compound Consumption by Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame . . . . . H-6
**TABLE H4** Feed and Compound Consumption by Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame . . . . . H-8

**TABLE H1**
**Feed and Compound Consumption by Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame**

| | 0 ppm | | 3,125 ppm | | | 6,250 ppm | | | 12,500 ppm | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Week | 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** | | | | | | | | | | | |
| 1-13 | 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 H1**
**Feed and Compound Consumption by Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame**

| | 25,000 ppm | | | 50,000 ppm | | |
|---|---|---|---|---|---|---|
| Week | 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** | | | | | | |
| 1-13 | 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 H2**
**Feed and Compound Consumption by Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame**

| | 0 ppm | | 3,125 ppm | | | 6,250 ppm | | | 12,500 ppm | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Week | 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** | | | | | | | | | | | |
| 1-13 | 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 H2**
**Feed and Compound Consumption by Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Aspartame**

| | 25,000 ppm | | | 50,000 ppm | | |
|---|---|---|---|---|---|---|
| Week | 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** | | | | | | |
| 1-13 | 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 H3**
**Feed and Compound Consumption by Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame**

| | 0 ppm | | 3,125 ppm | | | 6,250 ppm | | | 12,500 ppm | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Week | 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** | | | | | | | | | | | |
| 1-13 | 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 H3**
**Feed and Compound Consumption by Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame**

| | 25,000 ppm | | | 50,000 ppm | | |
|---|---|---|---|---|---|---|
| Week | 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** | | | | | | |
| 1-13 | 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 H4**
**Feed and Compound Consumption by Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame**

| | 0 ppm | | 3,125 ppm | | | 6,250 ppm | | | 12,500 ppm | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Week | 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** | | | | | | | | | | | |
| 1-13 | 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 H4**
**Feed and Compound Consumption by Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Aspartame**

| | 25,000 ppm | | | 50,000 ppm | | |
|---|---|---|---|---|---|---|
| Week | 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** | | | | | | |
| 1-13 | 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

# APPENDIX I
# 9-MONTH FEED STUDIES
# IN B6.129-Cdkn2a$^{tm1Rdp}$ (N2) DEFICIENT MICE


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# 9-MONTH FEED STUDY IN Cdkn2a DEFICIENT MICE

## INTRODUCTION

The original bioassay of aspartame by its manufacturer has been questioned due to the development of brain tumors in a few of the Sprague-Dawley rats that were exposed to the chemical. The objective of this study was to evaluate the potential carcinogenicity of aspartame administered in feed to Cdkn2a deficient mice for 9 months. The Cdkn2a deficient transgenic mouse line was selected for this study because Cdkn2a gene alterations are thought to play a role in human brain carcinogenesis (Liggett and Sidransky, 1998) and because these genetically altered animals have been proposed to be susceptible to glial cell tumors of the brain.

## MATERIALS AND METHODS

Aspartame was obtained from Spectrum Quality Products, Inc. (Gardena, CA), via Research Triangle Institute (RTI) (Research Triangle Park, NC), 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, RTI, and the study laboratory; 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 (Appendix G) The dose formulations were prepared every 2 weeks by mixing aspartame with feed. Further information on preparation and analysis of dose formulations may be found in Appendix G.

Groups of 15 male and 15 female B6.129-Cdkn2a$^{tm1Rdp}$ (N2) (Cdkn2a deficient) mice were fed diets containing 0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm aspartame for 40 weeks.

Male and female mice were obtained from Taconic Farms (Germantown, NY) for use in the 9-month studies. Animals were quarantined for 27 days and were 8 to 9 weeks old at the study start. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program; all results were negative. Mice were housed individually. Feed and water were available *ad libitum*. Feed consumption was measured weekly in all groups. 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 I1.

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 all mice. At necropsy, the brain, heart, right kidney, lungs, liver, 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 I1.

**TABLE I1**
**Experimental Design and Materials and Methods in the 9-Month Feed Study of Aspartame in Cdkn2a Deficient Mice**

**Study Laboratory**
BioReliance (Rockville, MD)

**Animal Source**
Taconic Farms, Inc. (Germantown, NY)

**Strain**
B6.129-Cdkn2a$^{tm1Rdp}$ mice heterozygous for the null allele of the Cdkn2a gene locus

**Time Held Before Studies**
27 days

**Average Age When Studies Began**
8-9 weeks

**Date of First Exposure**
October 27, 1999

**Duration of Exposure**
40 weeks

**Date of Last Exposure**
July 28, 2000

**Necropsy Dates**
July 26-28, 2000

**Average Age at Necropsy**
47-48 Weeks

**Size of Study Groups**
15 males and 15 females

**Method of Distribution**
Animals were distributed randomly into groups of approximately equal initial mean body weights.

**Animals per Cage**
1

**Method of Animal Identification**
Tail tattoo

**Diet**
Irradiated NTP-2000 open formula meal (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*

**Water**
Tap water (Washington Suburban Sanitary Commission Potomac Plant) via automatic watering system (Edstrom Industries, Inc. Waterford, WI), available *ad libitum*

**Cages**
Polycarbonate cages (Lab Products, Inc., Seaford, DE), changed weekly

**Bedding**
Irradiated Sani-Chips® (P.J. Murphy Forest Produces, Montville, NJ), changed weekly

**Table I1**
**Experimental Design and Materials and Methods in the 9-Month Feed Study of Aspartame in Cdkn2a Deficient Mice**

**Cage Filters**
Remay 2016 (Snow Filtration, West Chester, OH), changed every 2 weeks

**Racks**
Stainless steel (Lab Products, Inc., Seaford, DE), changed and rotated every 2 weeks

**Animal Room Environment**
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*

**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.

**Method of Sacrifice**
Carbon dioxide asphyxiation

**Necropsy**
Necropsies were performed on all mice. Organs weighed were the brain, heart, right kidney, liver, lungs, right testis, and thymus

**Histopathology**
Complete histopathology was performed on all mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, 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.

## Genetic Toxicology

The genetic toxicity of aspartame in Cdkn2a deficient mice was assessed by testing the ability of the chemical to induce mutations in micronucleated erythrocytes in peripheral blood. The protocols for these studies and the results are given in Appendix E.

## Results

Estimates of 9-month survival probabilities for male and female mice are shown in Table I2. Survival of all exposed groups was similar to that of the control group.

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 (Table I3); these decreases are likely related to variability of body weights within groups rather than a treatment effect. Mean body weights of female mice were similar to those of the controls throughout the study (Table I4).

There were no statistical differences found in absolute organ weights, but relative kidney weights of 6,250 and 25,000 ppm female mice were significantly decreased ($P<0.05$; data not shown).

**TABLE I2**
**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=1.000N | P=1.000N | 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**.

**Table I3**
**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 I3**
**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 I4**
**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 I4**
**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 | |

*Liver:* Minimal to mild cytoplasmic vacuolization of periportal hepatocytes was found in control and exposed male mice (Table I5). 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 treated 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 slightly increased; however, this increase was not significant (Table I5). 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 in the highest exposure group was not determined.

**Table I5**
**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

## Reference

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