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

NTP Genetically Modified Model Report on the

Toxicity Studies of Acesulfame Potassium (CASRN 55589-62-3) in FVB/N-TgN(v-*Ha-ras*)Led (Tg.AC) Hemizygous Mice and Carcinogenicity Studies of Acesulfame Potassium in B6.129-*Trp53*^{tm1Brd} (N5) Haploinsufficient Mice (Feed Studies)

NTP GMM 02

OCTOBER 2005

NTP REPORT

ON THE

TOXICITY STUDIES OF ACESULFAME POTASSIUM
(CAS NO. 55589-62-3)

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

AND CARCINOGENICITY STUDIES OF ACESULFAME POTASSIUM

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

(FEED STUDIES)

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

The studies described in this 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 Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection per se is not an indicator of a chemical's carcinogenic potential.

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

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CONTENTS

SUMMARY

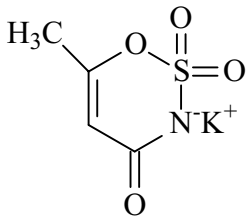
Background
Acesulfame potassium is an artificial sweetener widely used in beverages and foods. We tested if acesulfame potassium could cause cancer in two different strains of genetically modified mice.

Methods
We fed groups of male and female Tg.AC mice and male and female p53 mice diets containing up to 3% acesulfame potassium for 9 months. Animals given feed with no sweetener added served as the control groups. Tissues from 15 sites were examined for every animal.

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

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

ABSTRACT



ACESULFAME POTASSIUM

CAS No. 55589-62-3

Chemical Formula: $C_4H_4KNO_4S$ Molecular Weight: 201.25

Synonyms: ASK; HOE-095K; 6-methyl-1,2,3-oxathiazine-4(3-H)-one-2,2-dioxide potassium salt
Trade names: Sunette, Sweet One

Acesulfame potassium is an artificial sweetener used throughout the world in food and beverages. Acesulfame potassium was nominated by The Center for Science in the Public Interest because of its widespread use. Male and female Tg.AC hemizygous and p53 haploinsufficient mice were exposed to acesulfame potassium (at least 99% pure) in feed for 9 months. Genetic toxicology studies were conducted in 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%, 0.3%, 1%, or 3% acesulfame potassium (equivalent to average daily doses of approximately 420, 1,400, or 4,500 mg acesulfame potassium/kg body weight to males and 520, 1,700, or 5,400 mg/kg to females) for 40 weeks. Exposure to acesulfame potassium 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 acesulfame potassium.

9-Month Study in p53 Haploinsufficient Mice

Groups of 15 male and 15 female p53 haploinsufficient mice were fed diets containing 0%, 0.3%, 1%, or 3% acesulfame potassium (equivalent to average daily doses of approximately 475, 1,500, or 4,700 mg/kg to males and 570, 1,800, or 5,700 mg/kg to females) for 40 weeks. Exposure to acesulfame potassium 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 acesulfame potassium.

GENETIC TOXICOLOGY

Acesulfame potassium did not increase the frequency of micronucleated erythrocytes in peripheral blood of male or female Tg.AC hemizygous mice administered 0.3% to 3% in dosed feed. A similar study was conducted in p53 haploinsufficient mice, and a significant exposure concentration-related increase in the frequency of micronucleated erythrocytes was noted in males but not females.

CONCLUSIONS

Under the conditions of this 9-month feed study, there was *no evidence of carcinogenic activity** of acesulfame potassium in male or female p53 haploinsufficient mice exposed to 0.3%, 1%, or 3%.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 8. A summary of the Technical Report Review Subcommittee comments and public discussion on this technical report appears on page 9.

Summary of the 9-Month Carcinogenesis and Genetic Toxicology Studies of Acesulfame Potassium

	Male p53 Haploinsufficient Mice	Female p53 Haploinsufficient Mice
Concentrations in feed	0%, 0.3%, 1%, or 3% (0, 3,000, 10,000, or 30,000 ppm)	0%, 0.3%, 1%, or 3%
Body weights	Exposed groups similar to the control group	Exposed groups similar to the control group
Survival rates	14/15, 15/15, 15/15, 14/15	14/15, 14/15, 14/15, 14/15
Nonneoplastic effects	None	None
Neoplastic effects	None	None
Level of evidence of carcinogenic activity	No evidence	No evidence
Genetic toxicology		
Micronucleated erythrocytes		
Mouse peripheral blood *in vivo:*	Positive in p53 haploinsufficient males; negative in Tg.AC hemizygous males and females and p53 haploinsufficient 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 acesulfame potassium 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

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

Dr. R. Irwin, NIEHS, introduced the studies of acesulfame potassium in genetically modified mice by describing the uses of the sweetener, its nomination as a presumptive negative control for evaluation of the transgenic mouse models, the study design, and the survival, body weight, and histopathology observations. The proposed conclusion was:

Under the conditions of this 9-month feed study, there was *no evidence of carcinogenic activity* of acesulfame potassium in male or female p53 haploinsufficient mice exposed to 0.3%, 1%, or 3%. Because this is a new model, there is uncertainty whether the study possessed sufficient sensitivity to detect a carcinogenic effect.

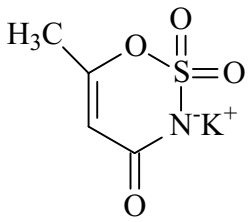
Dr. Storer, the first principal reviewer, agreed with the conclusions. He felt it more appropriate to characterize the Tg.AC mouse model as a zeta-globin promoter activation model with an oncogenic H-*ras* reporter phenotype than as a gain of oncogenic function H-*ras* model.

Dr. Elwell, the second principal reviewer, agreed with the conclusions and inquired if the mice might have been able to tolerate a higher dose. Dr. Irwin replied that in another study with CD-1 mice a mild weight gain depression was seen at this top dose.

Dr. Piegorsch, the third principal reviewer, also agreed with the conclusions.

Dr. Storer moved, and Dr. Elwell seconded, that the conclusions be accepted as written. The motion was then approved unanimously with eight votes.

INTRODUCTION



ACESULFAME POTASSIUM

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Synonyms: ASK; HOE-095K; 6-methyl-1,2,3-oxathiazine-4(3-H)-one-2,2-dioxide potassium salt
Trade names: Sunette, Sweet One

Chemical and Physical Properties

Acesulfame potassium is an odorless, colorless, crystalline powder approximately 200 times sweeter than sugar. Acesulfame potassium has a decomposition point of 225° C, is stable over a broad range of pH, and has a density of 1.81 g/cm^3 (*Merck Index,* 1996; HSDB, 2003; Parchem, 2003). Acesulfame potassium is also highly soluble in water, has a long shelf life, and is synergistic when used in combination with other sweeteners (Parchem, 2003).

Production, Use, and Human Exposure

Acesulfame potassium is an artificial sweetener used throughout the world. It is currently available as a dry powder for use in food and beverages and is present as a flavoring agent in numerous food items and diet drinks; therefore, there is extensive human exposure.

Absorption, Distribution, and Excretion

After oral administration, acesulfame potassium is rapidly and completely absorbed then rapidly eliminated in the urine of rats, dogs, and humans. After oral administration to rats, blood concentrations peaked within 30 minutes then declined with a $t_{1/2}$ of 4.8 hours. Following oral administration to humans (30 mg), blood levels peaked after 1 to 1.5 hours then declined with a $t_{1/2}$ of 2.5 hours.

Acesulfame potassium is rapidly distributed after oral administration and rapidly eliminated with no evidence of bioaccumulation in any tissue. At the time of maximum blood concentration, the highest levels were present in the gastrointestinal tract, bile, kidneys, and urinary bladder. Following oral administration of [^{14}C]-labeled acesulfame potassium to rats, 97.5% of the administered dose was excreted in the urine as parent compound within 24 hours; in humans, 98.4% was excreted in the

urine. Similar results were obtained for groups of rats dosed for 8 or 60 days prior to a histopathologic examination (Volz *et al.,* 1991).

TOXICITY

Experimental Animals

One published prechronic study of acesulfame potassium was found in the literature (Sinkeldam *et al.,* 1991a). Groups of 10 male and 10 female Wistar rats received acesulfame potassium in the diet at concentrations of 0%, 1%, 3%, or 10% for 13 weeks. Body weights and food and water consumption were measured. Urine samples were collected, and blood was drawn form the tip of the tail for hematology during week 13; blood was also collected at necropsy for determination of serum enzyme activities. At the end of the study, rats were necropsied, and adrenal gland, brain, cecal weights, heart, kidney, liver, ovary, spleen, testis, thymus, and thyroid gland weights were recorded. Tissues were collected, processed, and a histopathologic examination was conducted. There were no biologically significant differences in hematology or clinical chemistry parameters among the groups. There were slight decreases in mean relative spleen (males) and thymus (females) weights in rats that received 1% acesulfame potassium and in relative spleen weights of 3% females, which were small in magnitude and did not appear to be biologically significant. The only change that was biologically significant was the increase in relative cecal weights in males and females in the 10% groups. No exposure-related histologic lesions were observed.

Humans

No information on toxicity studies of acesulfame potassium in humans was found in the literature.

CARCINOGENICITY

Experimental Animals

Groups of 60 male and 60 female Wistar rats received diets that contained 0, 3,000, 10,000, or 30,000 ppm acesulfame potassium for 120 (males) or 123 (females) weeks (Sinkeldam *et al.,* 1991b). Body weights were recorded weeks 1 to 5, 8, 10, and 12, and at 4-week intervals thereafter. Feed consumption was measured at regular intervals throughout the study, and water consumption was measured during week 62 and 63. At weeks 33, 53, 78, 102, and 119, blood was collected from 10 males and 10 females in each group for hematology. Blood was drawn from 10 rats per group at weeks 34, 52, and 104 for blood urea nitrogen and glucose and from 10 rats per group for serum chemistry at weeks 34, 52, and 109. At weeks 35, 52, 78, and 104, urine was collected from 10 rats per group for urinalysis. Survival of all exposed groups was similar to the control groups. Mean body weights of 3,000 and 10,000 ppm males and females were similar to the controls throughout the study. The mean body weight of 30,000 ppm males was slightly lower than the controls up to week 80, after which there appeared to be no differences between any of the male groups. The mean body weight of 30,000 ppm females was lower than that of the controls, but the difference was no more than 5%. The only potential exposure-related response was the increased incidences of mammary gland tumors in females. The incidences of fibroadenoma were greater in exposed groups of females (43%, 48%, 48%) than that in control group (30%), and the incidences of adenocarcinoma were greater in exposed females (3%, 5%, 5%) than that in controls (2%). The authors concluded that the increased incidences of adenocarcinoma were not exposure-related because mammary gland adenocarcinomas are common in Wistar rats (historical control rate is 5%), and the incidence in the concurrent control group was relatively low compared to historical control data.

Groups of 100 male and 100 female Swiss mice received diets that contained 0, 3,000, 10,000, or 30,000 ppm acesulfame potassium for 80 weeks (Beems *et al.,* 1991). Body weights were recorded at weeks 1, 2, 4, and at 4-week intervals thereafter. Survival of all exposed groups was similar to that of the controls. Mean body weights of 30,000 ppm males and females were slightly lower than those of the other groups, but the differences were marginal. Mean liver weights of all exposed groups of males were less than that of the control group; kidney weights of exposed males and liver and kidney weights of exposed females were similar to the controls. No exposure-related lesions were observed.

Humans

No epidemiology studies of acesulfame potassium in humans were found in the literature.

GENETIC TOXICITY

Two studies measured cytogenetic effects in bone marrow cells of male Swiss albino mice administered acesulfame potassium by gavage. Mukherjee and Chakrabarti (1997) measured induction of chromosomal aberrations in mice treated with 15 to 2,250 mg/kg; significant increases in chromatid breaks were observed, and the increases in chromosomally abnormal cells were related to dose. A followup study by Mukhopadhyay *et al.* (2000) investigated induction of chromosomal aberrations in mice administered a mixture of acesulfame potassium (1.5, 15, and 150 mg/kg) and aspartame (3.5, 35, and 350 mg/kg); doses were chosen based on proposed limits of the two sweeteners together in soft drinks (150 ppm acesulfame potassium and 350 ppm aspartame); no induction of chromosomal aberrations was observed.

Jung *et al.* (1991) conducted a battery of genetic toxicity tests on acesulfame potassium. At doses from 4 to 10,000 µg/plate, acesulfame potassium failed to increase the frequency of revertants in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 with or without S9. Over the same dose range, no increase in the frequency point mutations leading to tryptophane independence was observed in *Escherichia coli* WP2uvrA. At concentrations of 10 to 1,000 µg/mL culture media, acesulfame potassium did not increase the frequency of azaguanine-resistant colonies of Chinese hamster V79 cells.

Incubation of freshly isolated hepatocytes from F344 rats for 20 hours in culture medium containing 25 to 5,000 µg/mL, acesulfame potassium did not increase incorporation of H3-thymidine into nuclear DNA, a measure of unscheduled DNA synthesis (Jung *et al.,* 1991).

No increase in the frequency of micronuclei was observed in the bone marrow of male or female NMRI mice after an acute gavage exposure to 0, 450, 1,500, or 4,500 mg acesulfame potassium/kg body weight (Jung *et al.,* 1991). There was no increase in the frequency of chromosomal aberrations in the bone marrow of male or female Chinese hamsters that received the same dose concentrations for 5 days.

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, two genetically altered mouse models with either a loss of heterozygosity in a critical cancer gene (Trp53) or a gain of oncogene function (Ha-*ras*) were used to determine how these animals would respond to acesulfame potassium exposure. These mouse models are 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 mutations 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 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 studies of acesulfame potassium and in the companion studies of aspartame (NTP, 2005) because this model has been reported to detect both nongenotoxic and genotoxic carcinogens (Spalding *et al.,* 1993; Tennant *et al.,* 1995*,* 1996; Pritchard *et al.,* 2003).

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

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

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

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

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

Study Rationale

Acesulfame potassium was nominated by The Center for Science in the Public Interest (CSPI) in 1996. At that time, the FDA was considering a food additive petition for the use of acesulfame potassium as an artificial sweetener in nonalcoholic beverages which would have substantially increased human exposure to acesulfame potassium. CSPI asked that the NTP review the data being submitted to support the petition, and if necessary, conduct additional testing or recommend to the FDA that additional testing be conducted by the sponsor. A review of the carcinogenicity data indicated that an

apparently adequate conventional 80-week mouse carcinogenicity study had been conducted with acesulfame potassium (Beems *et al.,* 1991). Because acesulfame potassium is not biotransformed after absorption and was negative in the 80-week mouse carcinogenicity study, it was considered a candidate for a negative control in the evaluation of Tg.AC and p53 mouse models.

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). 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, two models, the Tg.AC hemizygous (v-Ha-*ras*) and p53-deficient (p53 haploinsufficient) mice, have shown potential usefulness in identifying carcinogens (Pritchard *et al.,* 2003). Acesulfame potassium was one of the test agents selected for the continued evaluation of the genetically modified Tg.AC hemizygous and p53 haploinsufficient strains.

MATERIALS AND METHODS

Procurement and Characterization of Acesulfame Potassium

Acesulfame potassium was obtained from Riedel-deHaen (St. Louis, MO) via Research Triangle Institute (RTI) (Research Triangle Park, NC) in one lot (8415-76-02 RTI), which was used in the 9-month studies. Identity and purity analyses were conducted by the analytical chemistry laboratory, RTI, and the study laboratory; the study laboratory (BioReliance Corporation, Rockville, MD) also conducted stability analyses (Appendix F). Reports on analyses performed in support of the acesulfame potassium studies are on file at the National Institute of Environmental Health Sciences.

Lot 8415-76-02 RTI, a white, odorless, crystalline powder, was identified as acesulfame potassium by infrared and proton nuclear magnetic resonance spectroscopy. All spectra were consistent with the structure of acesulfame potassium. The purity of lot 8415-76-02 RTI was determined using high-performance liquid chromatography (HPLC) and confirmed by the study laboratory using major peak comparisons versus a reference sample from the same lot (stored at –20° C); HPLC indicated one major peak and no impurities. The purity was found to be greater than 99%.

To ensure stability, the bulk chemical was stored at room temperature under a headspace of inert gas, protected from light, in polyethylene bags inside plastic pails. Stability of the bulk chemical was monitored during the study by the study laboratory using HPLC. No degradation of the bulk chemical was observed.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared once every two to four weeks by mixing acesulfame potassium with feed (Table F1). Homogeneity studies of the 0.1% and 5% acesulfame potassium formulations and stability studies of the 0.1% formulations were performed by the analytical chemistry laboratory with HPLC. Homogeneity studies of the 0.3% and 3% formulations were performed by the study laboratory using HPLC. Homogeneity was confirmed, and stability was confirmed for at least 35 days for dose formulations stored in amber glass bottles at –20° C, –5° C, and 25° C, and when exposed to light and air for 7 days.

Periodic analyses of the dose formulations of acesulfame potassium were conducted by the study laboratory using HPLC. During the 9-month studies, the dose formulations were analyzed five times; all 15 of the dose formulations analyzed were within 10% of the target concentrations (Table F2). Animal room samples of these dose formulations were also analyzed, and 12 of the 15 samples were within 10% of the target concentrations (Table F2).

9-Month Studies

Study Design

Groups of 15 male and 15 female mice were fed diets containing 0%, 0.3%, 1%, or 3% (0, 3,000, 10,000, or 30,000 ppm) acesulfame potassium for 40 weeks.

Source and Specification of Animals

Male and female FVB/N-TgN(v-Ha-*ras*)Led (Tg.AC) hemizygous and B6.129-*Trp53*^{tm1Brd} (N5) haploinsufficient mice were obtained from Taconic Farms, Inc. (Germantown, NY), for use in the 9-month studies. Tg.AC hemizygous mice were quarantined for 11 days and p53 haploinsufficient mice were quarantined for 13 days before the beginning of the studies. Five male and five female mice in the control and 3% groups were randomly selected for parasite evaluation and gross observation of disease. Tg.AC hemizygous mice were 5 to 6 weeks old and p53 haploinsufficient mice were 6 to 7 weeks old at the beginning of the studies. The health of the mice 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 contamination. Cages were changed weekly, and racks were changed and rotated every 2 weeks. Further details of animal maintenance are given in Table 1.

Clinical Examinations and Pathology

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

Complete necropsies were performed on all mice, except the control positive mice treated with TPA. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Complete microscopic examinations were performed on the control and 3% groups; the lung was examined in all groups of male Tg.AC hemizygous mice. 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 evaluated by 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 and two NTP pathologists. Minimal discrepancies were identified and the final diagnoses represent a consensus of the three contractors and two NTP pathologists. Details of these review procedures have been described, in part, 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 Acesulfame Potassium

Tg.AC Hemizygous Mice	p53 Haploinsufficient Mice
Study Laboratory BioReliance Corporation (Rockville, MD)	BioReliance Corporation (Rockville, MD)
Strain FVB/N-TgN(v-Ha-*ras*)Led (Tg.AC) hemizygous	B6.129-*Trp53*^{tm1Brd} (N5) haploinsufficient
Animal Source Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies 11 days	13 days
Average Age When Studies Began 5 to 6 weeks	6 to 7 weeks
Date of First Exposure January 31, 2000 (males) February 1, 2000 (females)	February 2, 2000 (males) February 3, 2000 (females)
Duration of Exposure 40 weeks	40 weeks
Date of Last Exposure October 30-31, 2000	November 1-2, 2000
Necropsy Dates October 30-31, 2000	November 1-2, 2000
Average Age at Necropsy 44 to 45 weeks	45 to 46 weeks
Size of Study Groups 15 males and 15 females	15 males and 15 females
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Animals were distributed randomly into groups of approximately equal initial mean body weights.
Animals per Cage 1	1
Method of Animal Identification Tail tattoo	Tail tattoo
Diet Irradiated NTP-2000 open formula meal (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*	Same as Tg.AC hemizygous mouse study
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 mouse study

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

Tg.AC Hemizygous Mice	p53 Haploinsufficient Mice
Cages Polycarbonate cages (Lab Products, Inc., Seaford, DE), changed weekly	Same as Tg.AC hemizygous mouse study
Bedding Irradiated Sani-Chips® (P.J. Murphy Forest Produces, Montville, NJ), changed weekly	Same as Tg.AC hemizygous mouse study
Cage Filters Remay 2016 (Snow Filtration, West Chester, OH), changed every 2 weeks	Same as Tg.AC hemizygous mouse study
Racks Stainless steel (Lab Products, Inc., Seaford, DE), changed and rotated every 2 weeks	Same as Tg.AC hemizygous mouse study
Animal Room Environment Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Exposure Concentrations 0%, 0.3%, 1%, or 3% acesulfame potassium in feed, available *ad libitum*	0%, 0.3%, 1%, or 3% acesulfame potassium 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 Necropsy was performed on all mice.	Necropsy was performed on all mice.
Histopathology Complete histopathology was performed on all mice that died early and 0% and 3% 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 and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung and mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, tongue, trachea, urinary bladder, uterus, vagina, and Zymbal's gland. In addition, the lung was examined in the remaining groups of males.	Complete histopathology was performed on all mice that died early and 0% and 3% 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 and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung and mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, tongue, trachea, urinary bladder, uterus, vagina, and Zymbal's gland.

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, only to site-specific lesion free 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

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 acesulfame potassium was assessed by testing the ability of the chemical to induce increases in the frequency of micronucleated erythrocytes in mouse peripheral blood. The protocol for this study 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). Other tests, although less predictive of rodent carcinogenicity, 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 16 weeks. All males and 87% of the females developed more than 20 papillomas each by week 16 (data not shown). This is consistent with historical rates found in other studies (Tennant *et al.,* 2001).

Survival

Estimates of 9-month survival probabilities for male and female mice are shown in Table 2 and in the Kaplan-Meier survival curves (Figure 1). 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 Acesulfame Potassium

	0%	0.3%	1%	3%
Male				
Animals initially in study	15	15	15	15
Moribund	2	4	2	1
Animals surviving to study termination	13	11	13	14
Percent probability of survival at end of study[a]	87	73	87	93
Mean survival (days)[b]	258	249	268	267
Survival analysis[c]	P=0.430N	P=0.638	P=1.000N	P=0.951N
Female				
Animals initially in study	15	15	15	15
Moribund	1	0	2	2
Natural deaths	2	3	1	1
Animals surviving to study termination	12	12	12	12
Percent probability of survival at end of study	80	80	80	80
Mean survival (days)	266	255	267	262
Survival analysis	P=1.000	P=1.000	P=1.000	P=1.000

[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**.

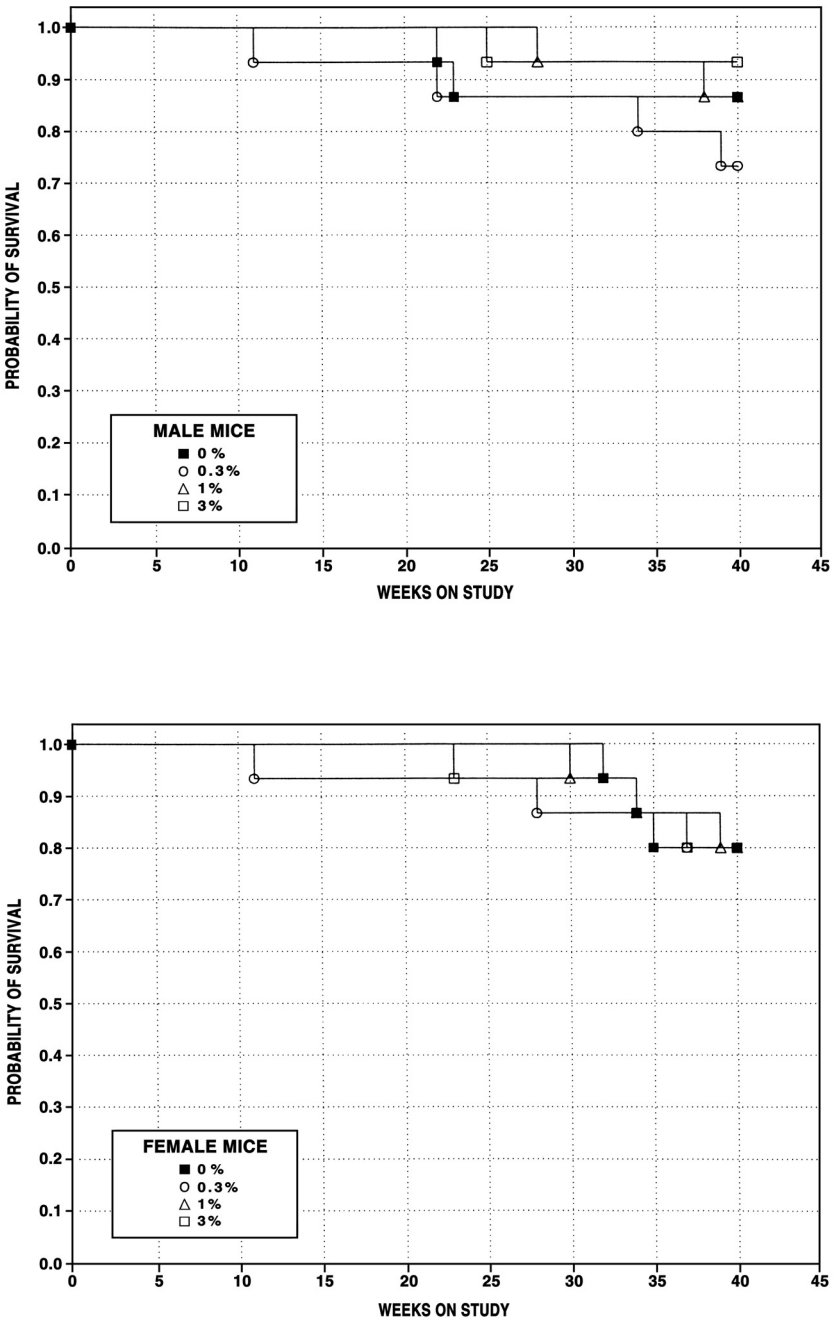

FIGURE 1
Kaplan-Meier Survival Curves for Male and Female Tg.AC Hemizygous Mice Exposed to Acesulfame Potassium in Feed for 9 Months

Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of all exposed groups of males were similar to those of the control group throughout the study; mean body weights of all exposed groups of females were less than the control group after week 35 (Tables 3 and 4; Figure 2). Because the body weights of females did not decrease with increasing exposure concentration, the body weight effect was not considered to be treatment related. Feed consumption by the exposed groups was similar to that by the control groups (Tables G1 and G2). Dietary concentrations of 0.3%, 1%, and 3% acesulfame potassium delivered average daily doses 420, 1,400, or 4,500 mg acesulfame potassium/kg body weight to males and 520, 1,700, or 5,400 mg/kg to females. Clinical findings included jaw masses, malocclusions, and cutaneous papillomas, but these findings did not occur in a pattern that would suggest a relationship to acesulfame potassium exposure.

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

Weeks on Study	0%		0.3%			1%			3%		
	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	21.2	15	21.2	100	15	21.3	101	15	21.3	101	15
2	22.6	15	22.0	97	15	22.7	100	15	22.5	100	15
3	23.3	15	23.6	101	15	23.7	102	15	23.7	102	15
4	24.2	15	24.9	103	15	24.8	103	15	24.7	102	15
5	24.9	15	25.7	103	15	25.3	102	15	25.1	101	15
6	25.5	15	26.3	103	15	26.4	104	15	25.6	100	15
7	26.3	15	26.5	101	15	26.8	102	15	26.9	102	15
8	25.9	15	27.2	105	15	27.5	106	15	27.4	106	15
9	27.4	15	27.6	101	15	27.7	101	15	27.7	101	15
10	27.5	15	27.6	100	15	28.0	102	15	27.7	101	15
11	27.0	15	28.2	104	14	28.4	105	15	27.6	102	15
12	28.0	15	29.0	104	14	29.2	104	15	28.8	103	15
13	28.2	15	28.9	103	14	29.4	104	15	29.0	103	15
14	28.8	15	29.8	104	14	29.7	103	15	29.3	102	15
15	29.3	15	29.9	102	14	30.0	102	15	29.9	102	15
16	29.5	15	29.8	101	14	30.3	103	15	30.1	102	15
17	29.6	15	29.8	101	14	30.5	103	15	30.4	103	15
18	29.2	15	30.5	105	14	30.7	105	15	30.6	105	15
19	29.4	15	30.4	103	14	30.5	104	15	30.7	104	15
20	29.5	15	29.7	101	14	30.5	103	15	30.6	104	15
21	29.5	15	30.1	102	14	31.2	106	15	31.1	105	15
22	29.5	15	30.9	105	14	31.6	107	15	31.5	107	15
23	30.4	14	32.0	105	13	31.4	103	15	31.8	105	15
24	31.5	13	31.1	99	13	31.8	101	15	31.9	101	15
25	31.6	13	32.2	102	13	32.2	102	15	32.1	102	15
26	31.8	13	32.2	101	13	32.2	101	15	32.8	103	14
27	32.0	13	32.2	101	13	32.2	101	15	32.3	101	14
28	32.0	13	32.6	102	13	32.9	103	15	32.8	103	14
29	32.4	13	32.8	101	13	33.1	102	14	32.5	100	14
30	32.5	13	33.3	103	13	33.4	103	14	33.0	102	14
31	32.5	13	33.3	103	13	33.1	102	14	33.0	102	14
32	32.5	13	33.2	102	13	33.6	103	14	33.7	104	14
33	32.3	13	32.5	101	13	33.2	103	14	33.0	102	14
34	32.2	13	32.7	102	13	33.4	104	14	33.4	104	14
35	32.6	13	32.8	101	12	33.4	103	14	33.4	103	14
36	33.0	13	32.7	99	12	33.7	102	14	33.4	101	14
37	32.7	13	33.5	102	12	34.1	104	14	33.3	102	14
38	32.9	13	33.5	102	12	33.4	102	14	33.2	101	14
39	33.2	13	32.9	99	12	34.4	104	13	33.9	102	14
40	33.2	13	33.6	101	11	34.1	103	13	33.5	101	14
Mean for weeks											
1-13	25.5		26.1	102		26.2	103		26.0	102	
14-40	31.3		31.9	102		32.2	103		32.1	103	

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

Weeks on Study	0%		0.3%			1%			3%		
	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	17.8	99	15	18.0	101	15	17.8	99	15
2	17.5	15	18.2	104	15	18.2	104	15	18.1	103	15
3	17.8	15	18.5	104	15	18.3	103	15	18.3	103	15
4	19.6	15	19.5	100	15	19.3	99	15	19.7	101	15
5	19.7	15	19.6	100	15	19.8	101	15	19.7	100	15
6	19.7	15	19.5	99	15	19.6	100	15	19.4	99	15
7	20.3	15	20.1	99	15	20.5	101	15	20.6	102	15
8	20.7	15	20.8	101	15	21.2	102	15	20.8	101	15
9	21.3	15	21.6	101	15	21.5	101	15	21.2	100	15
10	21.7	15	21.6	100	15	21.9	101	15	21.4	99	15
11	22.1	15	21.9	99	15	22.2	101	15	22.3	101	15
12	22.5	15	22.1	98	14	22.6	100	15	21.9	97	15
13	22.2	15	22.4	101	14	22.7	102	15	21.9	99	15
14	22.3	15	22.7	102	14	22.7	102	15	22.1	99	15
15	22.5	15	23.1	103	14	22.9	102	15	22.6	100	15
16	23.5	15	23.3	99	14	23.2	99	15	23.4	100	15
17	23.7	15	23.6	100	14	23.2	98	15	23.0	97	15
18	24.0	15	23.4	98	14	23.7	99	15	23.3	97	15
19	23.8	15	23.3	98	14	23.9	100	15	23.3	98	15
20	24.4	15	23.5	96	14	23.9	98	15	23.7	97	15
21	24.6	15	23.8	97	14	24.4	99	15	23.5	96	15
22	24.9	15	24.0	96	14	24.6	99	15	23.3	94	15
23	25.0	15	24.1	96	14	24.2	97	15	24.0	96	15
24	25.4	15	24.5	97	14	24.8	98	15	24.7	97	14
25	25.3	15	24.5	97	14	24.7	98	15	25.1	99	14
26	25.4	15	24.8	98	14	25.0	98	15	25.2	99	14
27	26.7	15	25.3	95	14	25.2	94	15	25.1	94	14
28	26.5	15	25.5	96	14	25.1	95	15	25.1	95	14
29	26.8	15	25.2	94	13	25.1	94	15	24.8	93	14
30	27.1	15	25.6	95	13	25.4	94	15	25.7	95	14
31	26.7	15	25.5	96	13	25.5	96	14	26.1	98	14
32	26.4	15	25.7	97	13	26.0	99	14	26.1	99	14
33	25.9	14	25.8	100	13	25.5	99	14	25.8	100	14
34	26.0	14	26.1	100	13	25.4	98	14	25.6	99	14
35	26.0	13	24.9	96	13	25.9	100	13	26.4	102	13
36	28.3	12	25.9	92	13	26.3	93	13	26.2	93	13
37	28.4	12	26.4	93	13	26.6	94	13	26.3	93	13
38	27.8	12	26.7	96	12	26.6	96	13	26.6	96	12
39	28.5	12	26.6	93	12	26.8	94	13	26.7	94	12
40	28.5	12	26.3	92	12	27.0	95	12	26.6	93	12
Mean for weeks											
1-13	20.2		20.3	100		20.4	101		20.2	100	
14-40	25.7		24.8	96		24.9	97		24.8	96	

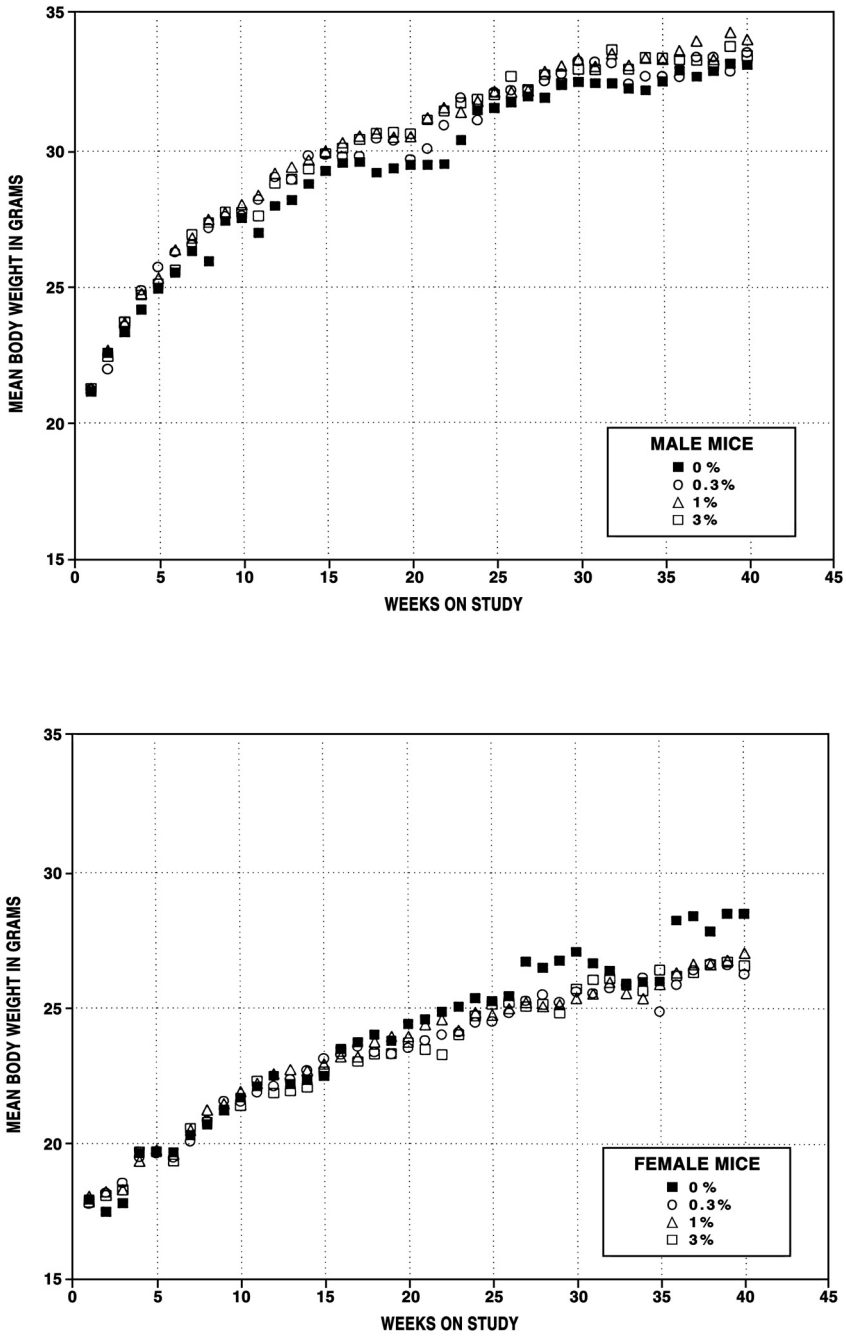

FIGURE 2
Growth Curves for Male and Female Tg.AC Hemizygous Mice Exposed to Acesulfame Potassium in Feed for 9 Months

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms of the jaw and skin. 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.

Jaw: Gross observations at necropsy included jaw masses in females. The incidence of odontogenic tumor in 1% females was slightly increased (0%, 2/15; 0.3%, 0/15; 1%, 6/15; 3%, 3/15; Table B3); however, the increased incidence was considered due to biologic variability and not to acesulfame potassium exposure.

Skin: Gross observations at necropsy included skin masses (papillomas) at several locations, particularly the face, head, and genital region; most masses occurred on the lip. Based on the microscopic evaluation, there were no significant increases in the incidences of squamous cell papilloma at any specific site in males or females (Tables A1 and B1). However, when papillomas at all sites were combined, there was an increase in the incidence of squamous cell papilloma in 1% males (3/15, 4/15, 8/15, 5/15; Table A3). With the exception of one male in the 3% group and one female in the 1% group, each of which had two papillomas at different locations, all other animals had a single papilloma (Tables A2 and B2).

9-Month Study in p53 Haploinsufficient Mice

Survival

Estimates of 9-month survival probabilities for male and female mice are shown in Table 5 and in the Kaplan-Meier survival curves (Figure 3). Survival of all exposed groups was similar to that of the control groups.

Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of 1% males were greater than those of the controls after week 26; however, this trend likely reflected biologic variability and sample size rather than a treatment effect (Table 6 and Figure 4). Mean body weights of 0.3% and 3% males and of all exposed groups of females were similar to those of the control groups (Table 7 and Figure 4). Feed consumption by the exposed groups was similar to that by the control groups (Tables G3 and G4). Dietary concentrations of 0.3%, 1%, and 3% acesulfame potassium delivered average daily doses of approximately 475, 1,500, or 4,700 mg/kg to males and 570, 1,800, or 5,700 mg/kg to females. There were no clinical findings related to acesulfame potassium exposure.

Table 5
Survival of p53 Haploinsufficient Mice in the 9-Month Feed Study of Acesulfame Potassium

	0%	0.3%	1%	3%
Male				
Animals initially in study	15	15	15	15
Moribund	1	0	0	1
Animals surviving to study termination	14	15	15	14
Percent probability of survival at end of study[a]	93	100	100	93
Mean survival (days)[b]	268	274	274	271
Survival analysis[c]	P=1.000	P=1.000N	P=1.000N	P=1.000N
Female				
Animals initially in study	15	15	15	15
Moribund	1	0	1	0
Natural deaths	0	1	0	1
Animals surviving to study termination	14	14	14	14
Percent probability of survival at end of study	93	93	93	93
Mean survival (days)	267	268	264	272
Survival analysis	P=1.000N	P=1.000N	P=1.000	P=1.000N

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

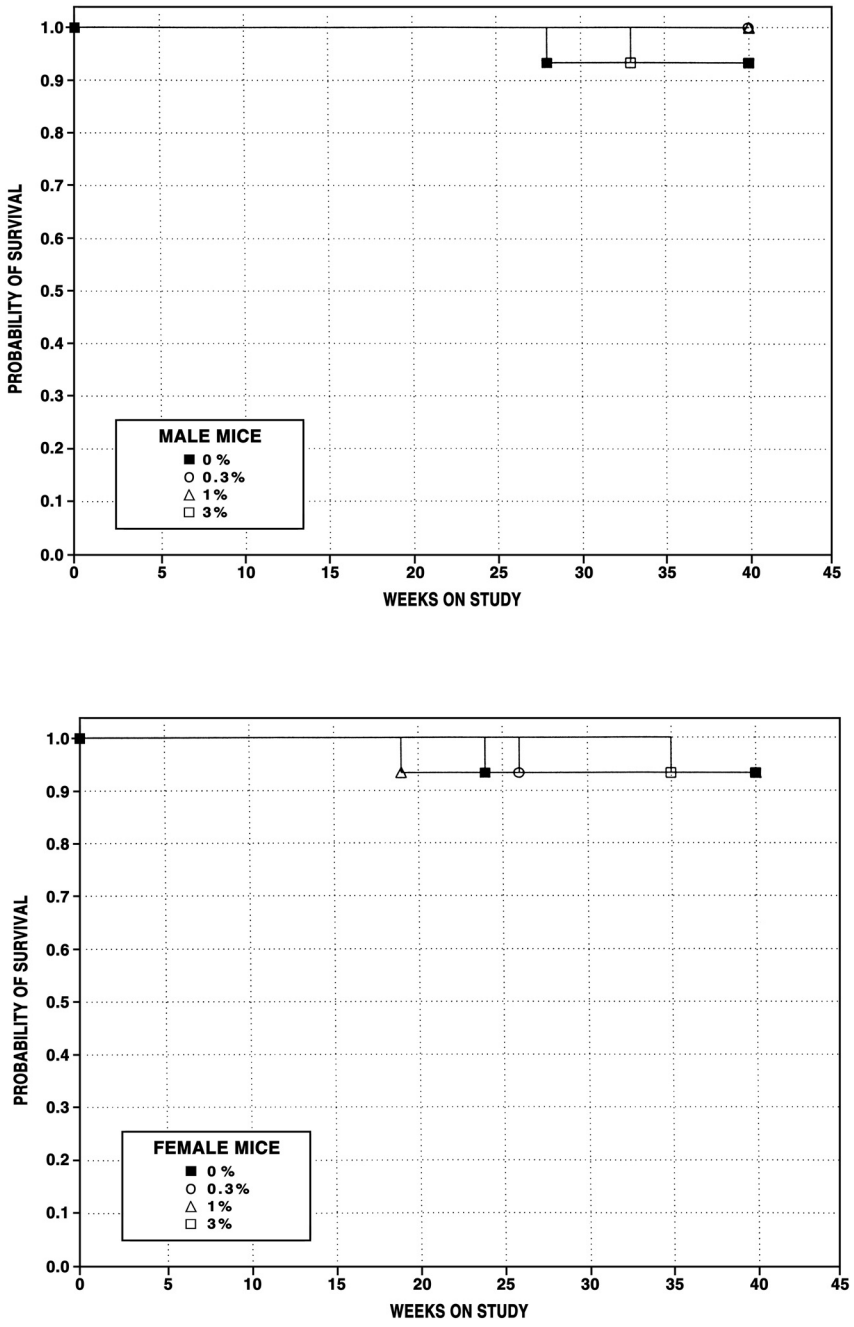

FIGURE 3
Kaplan-Meier Survival Curves for Male and Female p53 Haploinsufficient Mice Exposed to Acesulfame Potassium in Feed for 9 Months

TABLE 6
Mean Body Weights and Survival of Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Acesulfame Potassium

Weeks on Study	0%		0.3%			1%			3%		
	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	20.5	98	15	20.5	98	15	21.1	101	15
2	21.9	15	21.7	99	15	22.0	101	15	22.0	101	15
3	22.1	15	21.9	99	15	22.3	101	15	22.1	100	15
4	22.9	15	22.5	98	15	22.8	100	15	22.7	99	15
5	23.6	15	23.6	100	15	23.9	101	15	24.1	102	15
6	24.1	15	23.9	99	15	24.4	101	15	24.4	101	15
7	24.5	15	24.2	99	15	25.0	102	15	24.7	101	15
8	25.3	15	24.8	98	15	25.5	101	15	25.2	100	15
9	25.4	15	24.9	98	15	25.6	101	15	25.4	100	15
10	25.6	15	25.1	98	15	26.1	102	15	25.6	100	15
11	25.9	15	25.4	98	15	26.8	104	15	25.7	99	15
12	26.5	15	25.6	97	15	27.1	102	15	26.3	99	15
13	26.6	15	25.9	97	15	26.9	101	15	26.5	100	15
14	27.0	15	26.7	99	15	27.2	101	15	27.0	100	15
15	27.5	15	27.2	99	15	28.3	103	15	27.9	102	15
16	28.0	15	27.9	100	15	28.4	101	15	28.1	100	15
17	28.5	15	27.7	97	15	28.9	101	15	28.2	99	15
18	29.4	15	28.8	98	15	30.1	102	15	29.1	99	15
19	29.2	15	28.3	97	15	29.8	102	15	28.9	99	15
20	29.4	15	28.6	97	15	29.2	99	15	29.1	99	15
21	29.7	15	28.8	97	15	30.0	101	15	29.4	99	15
22	29.5	15	29.0	98	15	29.9	101	15	29.5	100	15
23	29.2	15	28.5	98	15	29.6	101	15	29.5	101	15
24	29.6	15	29.2	99	15	30.3	102	15	30.3	102	15
25	29.6	15	28.9	98	15	30.5	103	15	29.9	101	15
26	29.6	15	29.4	99	15	30.5	103	15	30.3	102	15
27	29.0	15	29.0	100	15	30.9	107	15	30.4	105	15
28	29.3	15	29.8	102	15	31.9	109	15	31.0	106	15
29	30.3	14	30.2	100	15	32.1	106	15	30.5	101	15
30	30.4	14	30.5	100	15	32.4	107	15	31.1	102	15
31	30.1	14	30.0	100	15	32.2	107	15	31.1	103	15
32	29.9	14	29.3	98	15	32.0	107	15	30.9	103	15
33	29.6	14	29.5	100	15	31.1	105	15	30.4	103	14
34	29.2	14	29.2	100	15	31.3	107	15	30.2	103	14
35	29.2	14	29.3	100	15	30.8	106	15	29.6	101	14
36	29.1	14	29.3	101	15	31.0	107	15	29.4	101	14
37	29.1	14	29.7	102	15	31.1	107	15	29.7	102	14
38	29.1	14	29.5	101	15	31.3	108	15	29.9	103	14
39	29.2	14	29.9	102	15	31.7	109	15	30.1	103	14
40	29.5	14	30.1	102	15	31.6	107	15	29.9	101	14
Mean for weeks											
1-13	24.3		23.8	98		24.5	101		24.3	100	
14-40	29.2		29.0	99		30.5	104		29.7	102	

TABLE 7
Mean Body Weights and Survival of Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Acesulfame Potassium

Weeks on Study	0%		0.3%			1%			3%		
	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	16.4	15	17.0	104	15	17.0	104	15	16.4	100	15
2	18.5	15	17.9	97	15	18.4	100	15	17.8	96	15
3	18.4	15	18.7	102	15	19.2	104	15	18.5	101	15
4	19.6	15	19.5	100	15	19.9	102	15	19.1	97	15
5	20.2	15	20.7	103	15	20.6	102	15	20.1	100	15
6	20.9	15	21.1	101	15	21.2	101	15	21.0	101	15
7	21.0	15	21.2	101	15	21.5	102	15	21.1	101	15
8	21.4	15	21.5	101	15	21.5	101	15	20.8	97	15
9	21.4	15	21.5	101	15	21.7	101	15	21.3	100	15
10	21.8	15	21.6	99	15	21.9	101	15	21.6	99	15
11	22.4	15	22.4	100	15	22.4	100	15	21.8	97	15
12	22.6	15	22.3	99	15	22.6	100	15	22.3	99	15
13	23.0	15	23.1	100	15	23.2	101	15	22.8	99	15
14	23.2	15	23.6	102	15	23.5	101	15	22.9	99	15
15	23.6	15	24.2	103	15	23.7	100	15	22.9	97	15
16	24.1	15	25.2	105	15	24.2	100	15	23.6	98	15
17	24.3	15	25.1	103	15	24.2	100	15	23.7	98	15
18	24.7	15	25.6	104	15	25.0	101	15	24.1	98	15
19	24.8	15	25.8	104	15	24.9	100	14	24.6	99	15
20	25.1	15	26.1	104	15	25.5	102	14	24.9	99	15
21	25.4	15	26.4	104	15	25.7	101	14	25.3	100	15
22	25.6	15	26.8	105	15	26.1	102	14	25.5	100	15
23	26.1	15	27.0	103	15	26.3	101	14	25.6	98	15
24	26.1	15	26.9	103	15	26.6	102	14	26.0	100	15
25	26.4	14	26.9	102	15	26.7	101	14	26.3	100	15
26	26.5	14	27.1	102	14	26.5	100	14	26.3	99	15
27	26.7	14	27.3	102	14	27.2	102	14	26.9	101	15
28	26.9	14	27.8	103	14	27.4	102	14	27.4	102	15
29	27.0	14	28.0	104	14	28.0	104	14	27.1	100	15
30	28.2	14	28.6	101	14	28.5	101	14	28.0	99	15
31	27.8	14	28.6	103	14	28.5	103	14	27.9	100	15
32	28.1	14	27.9	99	14	28.5	101	14	27.5	98	15
33	27.5	14	28.0	102	14	28.4	103	14	27.9	102	15
34	27.7	14	27.8	100	14	28.3	102	14	27.5	99	15
35	27.6	14	27.3	99	14	27.7	100	14	26.8	97	15
36	27.3	14	28.1	103	14	28.2	103	14	27.1	99	14
37	27.5	14	28.0	102	14	28.0	102	14	27.6	100	14
38	27.7	14	28.1	101	14	27.9	101	14	27.6	100	14
39	27.5	14	28.3	103	14	28.2	103	14	28.0	102	14
40	28.3	14	28.8	102	14	28.8	102	14	28.3	100	14
Mean for weeks											
1-13	20.6		20.7	100		20.9	101		20.4	99	
14-40	26.4		27.0	102		26.8	102		26.2	99	

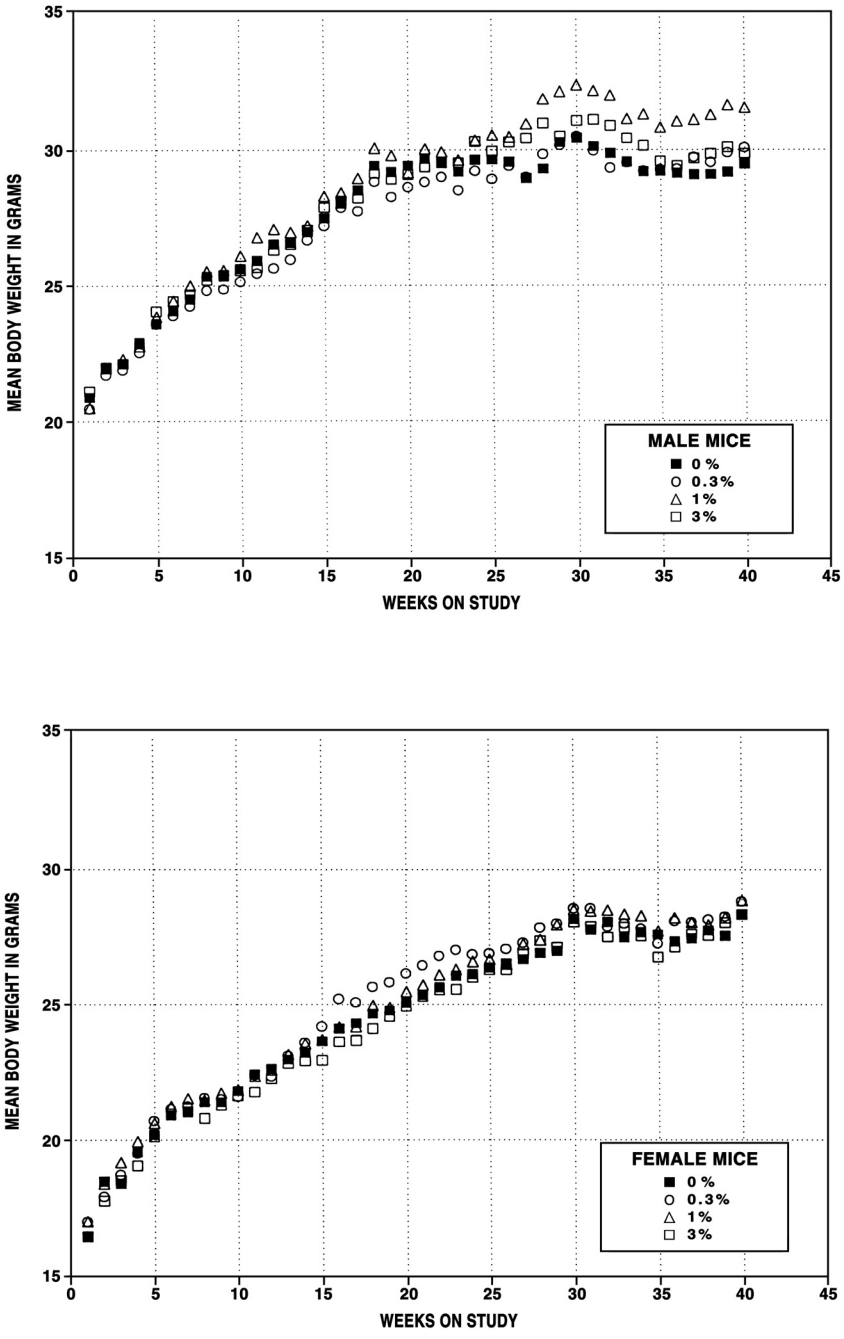

FIGURE 4
Growth Curves for Male and Female p53 Haploinsufficient Mice Exposed to Acesulfame Potassium in Feed for 9 Months

Pathology and Statistical Analyses

There were no neoplasms or nonneoplastic lesions in p53 haploinsufficient mice that were attributed to exposure to acesulfame potassium. 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

Acesulfame potassium did not increase the frequency of micronucleated normochromatic erythrocytes in peripheral blood of male or female Tg.AC hemizygous mice administered 0.3% to 3% of the chemical in feed for 9 months (Table E1). A similar study was conducted in p53 haploinsufficient mice, and in this study, a significant exposure-related increase in the frequency of micronucleated normochromatic erythrocytes was noted in males but not females; micronucleus frequencies in the 1% and 3% male groups were significantly elevated over the control group (Table E2). There was no significant alteration in the percentage of polychromatic erythrocytes among total erythrocytes in either Tg.AC hemizygous or p53 haploinsufficient mice.

DISCUSSION AND CONCLUSIONS

Acesulfame potassium is an oxathiazinone dioxide intense sweetener developed by Hoechst and currently marketed under the commercial names Sweet One and Sunett. Acesulfame is rapidly absorbed after oral administration but undergoes no biotransformation and is rapidly eliminated in the urine as parent compound within 24 hours of ingestion (Volz *et al.,* 1991). The carcinogenicity of acesulfame potassium has been evaluated in Wistar rats (Sinkeldam *et al.,* 1991a) and Swiss mice (Beems *et al.,* 1991). Both studies yielded negative results.

In the current studies, acesulfame potassium was evaluated in Tg.AC hemizygous and p53 haploinsufficent mice as a presumptive negative control to provide additional information regarding the use of these mice as alternative models for evaluation of carcinogenic activity. Specifically, these studies were designed to examine whether extending the study duration to 39 weeks to increase the opportunity for a positive response would lead to a significant increase in background tumors in control mice and to evaluate the forestomach in Tg.AC hemizygous mice as a possible target organ for papilloma induction for studies that use the oral route of administration.

No prechronic studies of acesulfame potassium were conducted by the NTP. Prechronic studies in rats conducted by Hoechst and the 80-week study in Swiss mice were used as a basis for dose selection in the current studies. During the 80-week mouse study, mean body weights of males and females that received 30,000 ppm acesulfame potassium in the diet were reduced, and mean liver weights of all exposed groups were significantly lower than those of the controls at the end of the study (Beems *et al.,* 1991). Based on these results, doses of 0.3%, 1%, and 3% (3,000, 10,000, or 30,000 ppm) administered in feed were selected for the current 9-month studies.

In the current studies, these exposure concentrations appeared to be well tolerated by both strains of mice. Survival and mean body weights of males and females were unaffected by exposure, and there were no exposure concentration-related clinical findings or other indications of toxicity. Therefore, it is possible that higher dietary concentrations may have been tolerated.

Cutaneous squamous cell papillomas occurred on the lip, prepuce, and pinna of exposed male Tg.AC hemizygous mice. Small epidermal squamous cell papillomas begin to occur at sites of chronic grooming (e.g., ears, nose, lips, paws, and the ano-urogenital areas) when the mice reach 20 to 26 weeks of age (Tenant *et al.,* 2001); however, the incidence is low (0% to 2%) in Tg.AC hemizygous mice and higher (up to 17%) in Tg.AC homozygous mice. The incidences in 34-week old control Tg.AC hemizygous mice was 3.7% in males and 3.8% in female (Mahler *et al.,* 1998). While historical control rates for 45-week old Tg.AC mice used in 39-week studies were not identified, they are expected to be higher; squamous cell papillomas occurred in 20% of the control males and females in the current study. Because spontaneous skin papillomas are relatively common in aging Tg.AC mice, especially in areas of grooming and/or licking, and the increase in the incidence of squamous cell papilloma occurred only in the 1% group of males with no increase in multiplicity, the slight increase was not considered related to acesulfame potassium.

The incidence of odontogenic tumors was slightly increased in Tg.AC hemizygous females that received 1% acesulfame potassium. The incidences of odontogenic tumors were not increased in any group of males or in females that received 3%. Therefore, the increase was not considered exposure related. Odontogenic tumors have occurred at incidences of 18% to 27% in control Tg.AC mice in 6-month studies (Tennant *et al.,* 2001).

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, 2003) there is a lack of acceptance within the scientific/regulatory community that a positive result in the Tg.AC model represents a true cancer response. Therefore, the confidence in this model to identify carcinogens appears to be less than that in either the p53 haploinsufficient or H*ras*2 model.

There were no increases in the incidences of neoplasms in p53 haploinsufficient mice exposed to acesulfame potassium. There was a significant increase in the frequency of micronucleated normochromatic erythrocytes at the end of the exposure period in males that received 1% or 3% acesulfame potassium. Although the increase was statistically significant, the actual magnitude of the increase was only 1 or 1.2 micronuclei/1,000 cells. Moreover, no increase was observed in females. In a review of the results of NTP prechronic and chronic studies in which peripheral blood micronuclei were evaluated in B6C3F$_1$ mice, Witt *et al.* (2000) considered a small increase in magnitude of response and/or a response in only one sex as a weak response and concluded that weak responses did not correlate with rodent carcinogenicity and were of uncertain biological significance. By contrast, a strong response (determined by magnitude of increase in frequency and response in males and females) strongly correlated with rodent carcinogenicity. The small increase in magnitude of response observed in male p53 haploinsufficient mice in the current study and the lack of response in females would classify this as a weak response and, therefore, is of uncertain biological significance.

In a study that examined micronuclei formation after acute exposure, Jung *et al.* (1991) administered 0, 450, 1,500, or 4,500 mg acesulfame potassium/kg body weight in distilled water by gavage to groups of 10 NMRI mice. Mice were dosed twice during a 24-hour period and then sacrificed 6 hours after the second dose; bone marrow was collected, and smears were prepared. Examination of 2,000 polychromatic erythrocytes per mouse revealed no increase in the frequency of micronuclei. The positive control, cyclophosphamide, produced a strong response.

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.

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 acesulfame potassium in the diet at concentrations up to 3% for 9 months.

CONCLUSIONS

Under the conditions of this 9-month feed study, there was *no evidence of carcinogenic activity** of acesulfame potassium in male or female p53 haploinsufficient mice exposed to 0.3%, 1%, or 3%.

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

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

	0%	0.3%	1%	3%
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund	2	4	2	1
Survivors				
Terminal sacrifice	13	11	13	14
Animals examined microscopically	15	15	15	15
Alimentary System				
Liver	(15)		(1)	(15)
Stomach, forestomach	(15)	(6)	(3)	(15)
Squamous cell papilloma	5 (33%)	4 (67%)	2 (67%)	6 (40%)
Tooth	(4)	(3)		(2)
Odontogenic tumor	4 (100%)	3 (100%)		1 (50%)
Cardiovascular System				
None				
Endocrine System				
Thyroid gland	(15)			(15)
Adenoma	1 (7%)			
General Body System				
None				
Genital System				
None				
Hematopoietic System				
Bone marrow	(15)			(15)
Spleen	(15)	(1)	(1)	(15)
Integumentary System				
Skin	(15)	(7)	(8)	(15)
Squamous cell papilloma	1 (7%)	1 (14%)	2 (25%)	4 (27%)
Dermis, mast cell tumor benign	1 (7%)			
Lip, squamous cell papilloma	2 (13%)	3 (43%)	5 (63%)	2 (13%)
Pinna, squamous cell papilloma			1 (13%)	
Prepuce, squamous cell papilloma	1 (7%)			
Musculoskeletal System				
None				

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

	0%	0.3%	1%	3%
Nervous System				
None				
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Alveolar/bronchiolar adenoma	1 (7%)	1 (7%)		2 (13%)
Special Senses System				
Harderian gland	(15)			(15)
Adenoma				1 (7%)
Urinary System				
None				
Systemic Lesions				
Multiple organs[b]	(15)	(15)	(15)	(15)
Leukemia erythrocytic			1 (7%)	1 (7%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	12	10	10	10
Total primary neoplasms	16	12	11	17
Total animals with benign neoplasms	9	8	9	9
Total benign neoplasms	12	9	10	15
Total animals with malignant neoplasms			1	1
Total malignant neoplasms			1	1
Total animals with uncertain neoplasms-benign or malignant	4	3		1
Total uncertain neoplasms	4	3		1

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

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

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

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

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

Number of Days on Study	1 4 8	1 5 7	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	
Carcass ID Number	0 1 2	0 0 6	0 0 1	0 0 2	0 0 3	0 0 4	0 0 5	0 0 7	0 0 8	0 0 9	0 1 0	0 1 1	0 1 3	0 1 4	0 1 5		Total Tissues/ Tumors
Hematopoietic System																	
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Lymph node, mandibular	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+		14
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	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		
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Squamous cell papilloma				X													1
Dermis, mast cell tumor benign										X							1
Lip, squamous cell papilloma				X				X									2
Prepuce, squamous cell papilloma														X			1
Musculoskeletal System																	
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Nervous System																	
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Respiratory System																	
Larynx	I	+	+	+	+	+	+	+	+	+	+	+	+	M	+		13
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Alveolar/bronchiolar adenoma												X					1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Special Senses System																	
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Zymbal's gland	M	+	+	+	+	+	+	M	M	M	M	M	I	+	M		7
Urinary System																	
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		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 Acesulfame Potassium: 0.3%

Number of Days on Study	071	148	233	271	274	274	274	274	274	274	274	274	274	274	274	274	
Carcass ID Number	030	022	022	023	016	017	018	019	020	022	025	026	027	028	029	02	Total Tissues/ Tumors
Alimentary System																	
Stomach, forestomach			+				+	+		+	+		+				6
Squamous cell papilloma			X				X			X			X				4
Tooth	+	+	+														3
Odontogenic tumor	X	X	X														3
Cardiovascular System																	
None																	
Endocrine System																	
None																	
General Body System																	
None																	
Genital System																	
Penis			+														1
Hematopoietic System																	
Spleen				+													1
Integumentary System																	
Skin				+	+	+		+		+				+	+		7
Squamous cell papilloma															X		1
Lip, squamous cell papilloma					X	X				X							3
Musculoskeletal System																	
None																	
Nervous System																	
None																	
Respiratory System																	
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Alveolar/bronchiolar adenoma												X					1
Special Senses System																	
None																	
Urinary System																	
Kidney				+													1
Systemic Lesions																	
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Acesulfame Potassium: 1%

Number of Days on Study	1 9 2	2 6 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	
Carcass ID Number	0 3 5	0 3 4	0 3 1	0 3 2	0 3 3	0 3 6	0 3 7	0 3 8	0 3 9	0 4 0	0 4 1	0 4 2	0 4 3	0 4 4	0 4 5		Total Tissues/ Tumors
Alimentary System																	
Liver	+																1
Stomach, forestomach				+		+							+				3
Squamous cell papilloma						X							X				2
Cardiovascular System																	
None																	
Endocrine System																	
None																	
General Body System																	
None																	
Genital System																	
Preputial gland			+														1
Hematopoietic System																	
Spleen	+																1
Integumentary System																	
Skin		+			+		+	+		+	+		+		+		8
Squamous cell papilloma													X		X		2
Lip, squamous cell papilloma					X		X	X		X	X						5
Pinna, squamous cell papilloma		X															1
Musculoskeletal System																	
None																	
Nervous System																	
None																	
Respiratory System																	
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Special Senses System																	
None																	
Urinary System																	
None																	
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 Acesulfame Potassium: 3%

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

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Acesulfame Potassium: 3%

Number of Days on Study	1 7 2	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	
Carcass ID Number	0 5 8	0 4 6	0 4 7	0 4 8	0 4 9	0 5 0	0 5 1	0 5 2	0 5 3	0 5 4	0 5 5	0 5 6	0 5 7	0 5 9	0 6 0	Total Tissues/ Tumors
Hematopoietic System																
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	14
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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Squamous cell papilloma							X	X					X	X		4
Lip, squamous cell papilloma				X										X		2
Musculoskeletal System																
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Nervous System																
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Respiratory System																
Larynx	I	+	+	+	I	+	+	M	+	+	M	I	+	I	I	8
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Alveolar/bronchiolar adenoma			X											X		2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Special Senses System																
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adenoma												X				1
Zymbal's gland	+	M	+	+	M	+	M	+	+	+	+	+	M	+	M	10
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Leukemia erythrocytic	X															1

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

	0%	0.3%	1%	3%
Lung: Alveolar/bronchiolar Adenoma				
Overall rate[a]	1/15 (7%)	1/15 (7%)	0/15 (0%)	2/15 (13%)
Adjusted rate[b]	7.5%	7.8%	0.0%	14.0%
Terminal rate[c]	1/13 (8%)	1/11 (9%)	0/13 (0%)	2/14 (14%)
First incidence (days)	274 (T)	274 (T)	—[e]	274 (T)
Poly-3 test[d]	P=0.351	P=0.752	P=0.487N	P=0.523
Skin: Squamous Cell Papilloma				
Overall rate	3/15 (20%)	4/15 (27%)	8/15 (53%)	5/15 (33%)
Adjusted rate	22.5%	31.4%	55.8%	35.1%
Terminal rate	3/13 (23%)	4/11 (36%)	7/13 (54%)	5/14 (36%)
First incidence (days)	274 (T)	274 (T)	264	274 (T)
Poly-3 test	P=0.429	P=0.473	P=0.075	P=0.381
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	5/15 (33%)	4/15 (27%)	2/15 (13%)	6/15 (40%)
Adjusted rate	37.5%	30.4%	14.1%	42.1%
Terminal rate	5/13 (39%)	3/11 (27%)	2/13 (15%)	6/14 (43%)
First incidence (days)	274 (T)	233	274 (T)	274 (T)
Poly-3 test	P=0.359	P=0.511N	P=0.164N	P=0.554
Tooth: Odontogenic Tumor				
Overall rate	4/15 (27%)	3/15 (20%)	0/15 (0%)	1/15 (7%)
Adjusted rate	26.7%	20.0%	0.0%	7.0%
Terminal rate	2/13 (15%)	0/11 (0%)	0/13 (0%)	1/14 (7%)
First incidence (days)	148	71	—	274 (T)
Poly-3 test	P=0.118N	P=0.501N	P=0.051N	P=0.179N
All Organs: Erythrocytic Leukemia				
Overall rate	0/15 (0%)	0/15 (0%)	1/15 (7%)	1/15 (7%)
Adjusted rate	0.0%	0.0%	6.7%	6.7%
Terminal rate	0/13 (0%)	0/11 (0%)	0/13 (0%)	0/14 (0%)
First incidence (days)	—	—[f]	192	172
Poly-3 test	P=0.352	—	P=0.522	P=0.523
All Organs: Benign Neoplasms				
Overall rate	9/15 (60%)	8/15 (53%)	9/15 (60%)	9/15 (60%)
Adjusted rate	67.4%	60.9%	62.7%	63.2%
Terminal rate	9/13 (69%)	7/11 (64%)	8/13 (62%)	9/14 (64%)
First incidence (days)	274 (T)	233	264	274 (T)
Poly-3 test	P=0.561N	P=0.521N	P=0.555N	P=0.564N
All Organs: Malignant Neoplasms				
Overall rate	0/15 (0%)	0/15 (0%)	1/15 (7%)	1/15 (7%)
Adjusted rate	0.0%	0.0%	6.7%	6.7%
Terminal rate	0/13 (0%)	0/11 (0%)	0/13 (0%)	0/14 (0%)
First incidence (days)	—	—	192	172
Poly-3 test	P=0.352	—	P=0.522	P=0.523

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

	0%	0.3%	1%	3%
All Organs: Benign or Malignant Neoplasms				
Overall rate	12/15 (80%)	10/15 (67%)	10/15 (67%)	10/15 (67%)
Adjusted rate	80.0%	66.8%	66.7%	66.7%
Terminal rate	10/13 (77%)	7/11 (64%)	8/13 (62%)	9/14 (64%)
First incidence (days)	148	71	192	172
Poly-3 test	P=0.392N	P=0.346N	P=0.343N	P=0.343N

(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 Acesulfame Potassium[a]

	0%	0.3%	1%	3%
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund	2	4	2	1
Survivors				
Terminal sacrifice	13	11	13	14
Animals examined microscopically	15	15	15	15
Alimentary System				
Liver	(15)		(1)	(15)
Hematopoietic cell proliferation	1 (7%)			
Inflammation, chronic	1 (7%)			
Hepatocyte, necrosis, focal	1 (7%)			
Mesentery	(2)			(2)
Fat, necrosis, focal	2 (100%)			2 (100%)
Pancreas	(15)			(15)
Inflammation, chronic active	1 (7%)			
Cardiovascular System				
None				
Endocrine System				
Adrenal cortex	(14)			(15)
Atrophy	10 (71%)			11 (73%)
Hypertrophy, focal	9 (64%)			5 (33%)
General Body System				
None				
Genital System				
Preputial gland	(15)		(1)	(15)
Cyst	1 (7%)		1 (100%)	
Testes	(15)			(15)
Germinal epithelium, degeneration, focal	1 (7%)			1 (7%)

[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 Acesulfame Potassium

	0%	0.3%	1%	3%
Hematopoietic System				
Bone marrow	(15)			(15)
Hyperplasia				1 (7%)
Lymph node, mandibular	(14)			(15)
Hyperplasia	2 (14%)			2 (13%)
Spleen	(15)	(1)	(1)	(15)
Atrophy	1 (7%)			
Hematopoietic cell proliferation	10 (67%)	1 (100%)		7 (47%)
Thymus	(15)			(14)
Atrophy, diffuse	3 (20%)			2 (14%)
Atrophy, focal	2 (13%)			
Integumentary System				
Skin	(15)	(7)	(8)	(15)
Sebaceous gland, hyperplasia, focal	1 (7%)			
Subcutaneous tissue, pinna, inflammation, acute, focal		1 (14%)		
Musculoskeletal System				
None				
Nervous System				
None				
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Alveolar epithelium, alveolus, inflammation, chronic active, focal				1 (7%)
Nose	(15)			(15)
Glands, hyperplasia	1 (7%)			
Special Senses System				
Eye	(15)			(15)
Retina, atrophy	15 (100%)			14 (93%)
Urinary System				
Kidney	(15)	(1)		(15)
Infiltration cellular, plasma cell		1 (100%)		
Renal tubule, dilatation, diffuse				1 (7%)
Renal tubule, dilatation, focal				2 (13%)
Urinary bladder	(15)			(15)
Edema	1 (7%)			

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

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

	0%	0.3%	1%	3%
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund	1		2	2
Natural deaths	2	3	1	1
Survivors				
Terminal sacrifice	12	12	12	12
Animals examined microscopically	15	14	14	15
Alimentary System				
Liver	(15)	(2)		(15)
Stomach, forestomach	(14)	(9)	(10)	(14)
Squamous cell papilloma	7 (50%)	6 (67%)	9 (90%)	8 (57%)
Squamous cell papilloma, multiple	2 (14%)	1 (11%)		
Tongue	(15)	(1)		(15)
Squamous cell carcinoma, metastatic, uncertain primary site	1 (7%)			
Tooth	(2)	(1)	(6)	(4)
Odontogenic tumor	2 (100%)		6 (100%)	3 (75%)
Cardiovascular System				
None				
Endocrine System				
Pituitary gland	(12)			(14)
General Body System				
None				
Genital System				
Uterus	(13)	(2)	(1)	(14)
Sarcoma stromal				1 (7%)
Hematopoietic System				
Spleen	(15)		(3)	(15)
Integumentary System				
Skin	(15)	(5)	(6)	(15)
Squamous cell papilloma	1 (7%)	2 (40%)	3 (50%)	1 (7%)
Lip, squamous cell papilloma			2 (33%)	
Vulva, squamous cell papilloma	2 (13%)	2 (40%)	2 (33%)	3 (20%)

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

	0%	0.3%	1%	3%
Musculoskeletal System				
None				
Nervous System				
None				
Respiratory System				
Lung	(15)			(15)
Alveolar/bronchiolar adenoma	1 (7%)			1 (7%)
Special Senses System				
Harderian gland	(14)	(1)		(15)
Urinary System				
Kidney	(15)	(1)		(15)
Urinary bladder	(13)			(15)
Transitional epithelium, papilloma				1 (7%)
Systemic Lesions				
Multiple organs	(15)	(14)	(14)	(15)
Leukemia erythrocytic	2 (13%)	1 (7%)		1 (7%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	12	10	13	13
Total primary neoplasms	17	12	22	19
Total animals with benign neoplasms	11	9	10	10
Total benign neoplasms	13	11	16	14
Total animals with malignant neoplasms	2	1		2
Total malignant neoplasms	2	1		2
Total animals with metastatic neoplasms	1			
Total metastatic neoplasms	1			
Total animals with malignant neoplasms of uncertain primary site	1			
Total animals with uncertain neoplasms-benign or malignant	2		6	3
Total uncertain neoplasms	2		6	3

[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 Acesulfame Potassium: 0%

Number of Days on Study	224	236	240	274	274	274	274	274	274	274	274	274	274	274	274	
Carcass ID Number	07 79	07 77	07 76	07 78	08 80	08 81	08 82	08 83	08 84	08 85	08 86	08 87	08 88	08 89	09 90	Total Tissues/ Tumors
Alimentary System																
Esophagus	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Gallbladder	A	A	+	+	+	+	+	+	+	+	M	+	+	+	+	12
Intestine large, colon	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Intestine large, rectum	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Intestine large, cecum	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Intestine small, duodenum	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Intestine small, jejunum	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Intestine small, ileum	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Mesentery						+			+							2
Pancreas	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Salivary glands	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Stomach, forestomach	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Squamous cell papilloma				X			X		X	X	X		X	X		7
Squamous cell papilloma, multiple	X														X	2
Stomach, glandular	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Squamous cell carcinoma, metastatic, uncertain primary site			X													1
Tooth		+						+								2
Odontogenic tumor		X						X								2
Cardiovascular System																
Blood vessel	+	+	+	+	+	M	+	+	+	+	+	M	M	+	+	12
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Adrenal medulla	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Islets, pancreatic	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Parathyroid gland	M	A	+	M	M	+	+	M	+	+	M	M	M	M	M	5
Pituitary gland	+	I	+	+	+	+	+	I	I	+	+	+	+	+	+	12
Thyroid gland	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	14
General Body System																
None																
Genital System																
Clitoral gland	M	A	+	+	+	+	+	M	I	+	+	M	+	M	+	9
Ovary	A		+	+	+	+	+	+	+	+	+	+	+	+	+	13
Uterus	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Vagina	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	13

+: 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 Acesulfame Potassium: 0%

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

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Acesulfame Potassium: 0.3%

Number of Days on Study	0 7 7	1 9 6	2 5 6	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	
Carcass ID Number	1 0 3	0 9 9	1 0 1	0 9 1	0 9 2	0 9 3	0 9 4	0 9 5	0 9 6	0 9 7	0 9 8	1 0 0	1 0 2	1 0 4	1 0 5		Total Tissues/ Tumors
Alimentary System																	
Esophagus	+																1
Gallbladder	A																
Intestine large, colon	A																
Intestine large, rectum	A																
Intestine large, cecum	A																
Intestine small, duodenum	A																
Intestine small, jejunum	A																
Intestine small, ileum	A																
Liver	+	+															2
Pancreas	+																1
Salivary glands	+																1
Stomach, forestomach	A	A		+	+			+	+	+	+	+	+		+		9
Squamous cell papilloma				X	X			X	X				X		X		6
Squamous cell papilloma, multiple												X					1
Stomach, glandular	A																
Tongue	+																1
Tooth			+														1
Cardiovascular System																	
Blood vessel	+																1
Heart	A																
Endocrine System																	
Adrenal cortex	+																1
Adrenal medulla	+																1
Islets, pancreatic	+																1
Parathyroid gland	M																
Pituitary gland	M																
Thyroid gland	A																
General Body System																	
None																	
Genital System																	
Clitoral gland	+																1
Ovary	A		A											+			1
Uterus	A					+		+									2
Vagina	A																
Hematopoietic System																	
Bone marrow	+																1
Lymph node, mandibular	+																1
Lymph node, mesenteric	A																
Spleen	A																
Thymus	+																1

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Acesulfame Potassium: 0.3%

Number of Days on Study	0 7 7	1 9 6	2 5 6	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	
Carcass ID Number	1 0 3	0 9 9	1 0 1	0 9 1	0 9 2	0 9 3	0 9 4	0 9 5	0 9 6	0 9 7	0 9 8	1 0 0	1 0 2	1 0 4	1 0 5	Total Tissues/ Tumors
Integumentary System																
Mammary gland	+															1
Skin	+			+	+						+			+		5
Squamous cell papilloma					X						X					2
Vulva, squamous cell papilloma				X										X		2
Musculoskeletal System																
Bone	+		+													2
Skeletal muscle	+															1
Nervous System																
Brain	+															1
Respiratory System																
Larynx	+															1
Lung	A															
Nose	+															1
Trachea	+															1
Special Senses System																
Eye	A															
Harderian gland	+															1
Zymbal's gland	+															1
Urinary System																
Kidney	+															1
Urinary bladder	A		A													
Systemic Lesions																
Multiple organs	+	+	+	+	+	+		+	+	+	+	+	+	+	+	14
Leukemia erythrocytic		X														1

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Acesulfame Potassium: 1%

Number of Days on Study	207	232	270	274	277	274	274	277	274	274	274	274	274	274	274	
Carcass ID Number	11106	11161	11163	11206	11007	11208	11009	11011	11112	11114	11115	11117	11118	11119	11120	Total Tissues/ Tumors
Alimentary System																
Mesentery			+													1
Stomach, forestomach		+	+	+	+	+	+			+	+	+		+		10
Squamous cell papilloma		X	X	X	X	X	X				X	X		X		9
Tooth	+	+			+		+	+	+							6
Odontogenic tumor	X	X			X		X	X	X							6
Cardiovascular System																
None																
Endocrine System																
None																
General Body System																
None																
Genital System																
Uterus					+											1
Hematopoietic System																
Spleen			+						+			+				3
Integumentary System																
Skin					+	+					+	+		+	+	6
Squamous cell papilloma					X							X		X		3
Lip, squamous cell papilloma											X			X		2
Vulva, squamous cell papilloma						X									X	2
Musculoskeletal System																
Bone						+										1
Nervous System																
None																
Respiratory System																
None																
Special Senses System																
None																
Urinary System																
None																
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+		+	+	14

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Acesulfame Potassium: 3%

Number of Days on Study	1 5 6	2 3 2	2 5 5	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	
Carcass ID Number	1 2 4	1 2 1	1 2 9	1 2 2	1 2 3	1 2 5	1 2 6	1 2 7	1 2 8	1 3 0	1 3 1	1 3 2	1 3 3	1 3 4	1 3 5	Total Tissues/ Tumors
Alimentary System																
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Gallbladder	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	14
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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	14
Squamous cell papilloma					X	X		X	X			X	X	X	X	8
Stomach, glandular	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	14
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Tooth	+	+	+										+			4
Odontogenic tumor	X	X											X			3
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	+	+	6
Pituitary gland	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	14
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
None																
Genital System																
Clitoral gland	+	M	+	+	+	+	+	+	+	M	I	+	+	+	+	12
Ovary	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	14
Uterus	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	14
Sarcoma stromal															X	1
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	14
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	14

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Acesulfame Potassium: 3%

Number of Days on Study	1 5 6	2 3 2	2 5 5	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	
Carcass ID Number	1 2 4	1 2 1	1 2 9	1 2 2	1 2 3	1 2 5	1 2 6	1 2 7	1 2 8	1 3 0	1 3 1	1 3 2	1 3 3	1 3 4	1 3 5	Total Tissues/ Tumors
Integumentary System																
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Squamous cell papilloma				X												1
Vulva, squamous cell papilloma									X			X	X			3
Musculoskeletal System																
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Nervous System																
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Respiratory System																
Larynx	+	I	+	I	+	I	+	+	+	I	+	+	+	+	+	11
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Alveolar/bronchiolar adenoma										X						1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Special Senses System																
Ear											+				+	2
Eye	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	14
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Zymbal's gland	+	+	+	M	+	+	+	+	M	M	I	M	+	+	+	10
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Transitional epithelium, papilloma															X	1
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 Acesulfame Potassium

	0%	0.3%	1%	3%
Lung: Alveolar/bronchiolar Adenoma				
Overall rate[a]	1/15 (7%)	0/0	0/0	1/15 (7%)
Adjusted rate[b]	7.2%	0.0%	0.0%	7.4%
Terminal rate[c]	1/12 (8%)	0/0	0/0	1/12 (8%)
First incidence (days)	274 (T)	—[e]	—	274 (T)
Poly-3 test[d]	—[f]	—	—	P=0.757
Skin: Squamous Cell Papilloma				
Overall rate	3/15 (20%)	4/15 (27%)	6/15 (40%)	4/15 (27%)
Adjusted rate	21.1%	30.3%	42.9%	29.4%
Terminal rate	2/12 (17%)	4/12 (33%)	6/12 (50%)	4/12 (33%)
First incidence (days)	236	274 (T)	274 (T)	274 (T)
Poly-3 test	P=0.489	P=0.456	P=0.201	P=0.473
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	9/15 (60%)	7/15 (47%)	9/15 (60%)	8/15 (53%)
Adjusted rate	62.9%	53.0%	62.4%	58.8%
Terminal rate	8/12 (67%)	7/12 (58%)	7/12 (58%)	8/12 (67%)
First incidence (days)	224	274 (T)	232	274 (T)
Poly-3 test	P=0.581N	P=0.446N	P=0.637N	P=0.567N
Tooth: Odontogenic Tumor				
Overall rate	2/15 (13%)	0/15 (0%)	6/15 (40%)	3/15 (20%)
Adjusted rate	14.1%	0.0%	40.1%	20.3%
Terminal rate	1/12 (8%)	0/12 (0%)	4/12 (33%)	1/12 (8%)
First incidence (days)	236	—	207	156
Poly-3 test	P=0.319	P=0.248N	P=0.120	P=0.519
All Organs: Erythrocytic Leukemia				
Overall rate	2/15 (13%)	1/15 (7%)	0/15 (0%)	1/15 (7%)
Adjusted rate	13.6%	7.2%	0.0%	7.3%
Terminal rate	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/12 (0%)
First incidence (days)	224	196	—	255
Poly-3 test	P=0.501N	P=0.521N	P=0.242N	P=0.522N
All Organs: Benign Neoplasms				
Overall rate	11/15 (73%)	9/15 (60%)	10/15 (67%)	10/15 (67%)
Adjusted rate	75.0%	68.2%	69.3%	73.5%
Terminal rate	9/12 (75%)	9/12 (75%)	8/12 (67%)	10/12 (83%)
First incidence (days)	224	274 (T)	232	274 (T)
Poly-3 test	P=0.569	P=0.509N	P=0.528N	P=0.635N
All Organs: Malignant Neoplasms				
Overall rate	3/15 (20%)	1/15 (7%)	0/15 (0%)	2/15 (13%)
Adjusted rate	20.0%	7.2%	0.0%	14.5%
Terminal rate	0/12 (0%)	0/12 (0%)	0/12 (0%)	1/12 (8%)
First incidence (days)	224	196	—	255
Poly-3 test	P=0.623N	P=0.329N	P=0.119N	P=0.540N

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

	0%	0.3%	1%	3%
All Organs: Benign or Malignant Neoplasms				
Overall rate	13/15 (87%)	10/15 (67%)	13/15 (87%)	13/15 (87%)
Adjusted rate	86.7%	72.3%	86.7%	86.7%
Terminal rate	10/12 (83%)	9/12 (75%)	10/12 (83%)	10/12 (83%)
First incidence (days)	224	196	207	156
Poly-3 test	P=0.443	P=0.311N	P=0.698	P=0.698

(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 Acesulfame Potassium[a]

	0%	0.3%	1%	3%
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund	1		2	2
Natural deaths	2	3	1	1
Survivors				
Terminal sacrifice	12	12	12	12
Animals examined microscopically	15	14	14	15
Alimentary System				
Liver	(15)	(2)		(15)
Inflammation, chronic	2 (13%)			3 (20%)
Necrosis, focal	1 (7%)			
Tension lipidosis, focal				1 (7%)
Hepatocyte, inflammation, chronic	1 (7%)			
Hepatocyte, necrosis, focal	2 (13%)			3 (20%)
Mesentery	(2)		(1)	
Fat, inflammation, acute, focal			1 (100%)	
Fat, necrosis, focal	2 (100%)			
Tongue	(15)	(1)		(15)
Ulcer	1 (7%)			
Cardiovascular System				
None				
Endocrine System				
Adrenal cortex	(14)	(1)		(15)
Subcapsular, hyperplasia, focal	9 (64%)			6 (40%)
Zona reticularis, vacuolization cytoplasmic	9 (64%)			10 (67%)
General Body System				
None				
Genital System				
Uterus	(13)	(2)	(1)	(14)
Endometrium, hyperplasia, cystic	7 (54%)	2 (100%)	1 (100%)	10 (71%)
Vagina	(13)			(15)
Hyperplasia	1 (8%)			

[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 Acesulfame Potassium

	0%	0.3%	1%	3%
Hematopoietic System				
Lymph node, mandibular	(12)	(1)		(15)
Hyperplasia	1 (8%)			3 (20%)
Spleen	(15)		(3)	(15)
Hematopoietic cell proliferation	12 (80%)		3 (100%)	13 (87%)
Pigmentation	3 (20%)			2 (13%)
Thymus	(11)	(1)		(14)
Atrophy, diffuse	1 (9%)			1 (7%)
Integumentary System				
None				
Musculoskeletal System				
Bone	(14)	(2)	(1)	(15)
Mandible, inflammation, chronic active		1 (50%)		1 (7%)
Nervous System				
None				
Respiratory System				
Lung	(15)			(15)
Hemorrhage, focal				1 (7%)
Special Senses System				
Eye	(13)			(14)
Retina, atrophy	13 (100%)			14 (100%)
Harderian gland	(14)	(1)		(15)
Infiltration cellular, focal, lymphocyte	1 (7%)			
Urinary System				
Kidney	(15)	(1)		(15)
Renal tubule, dilatation, focal				1 (7%)

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

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

	0%	0.3%	1%	3%
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund	1			1
Survivors				
Terminal sacrifice	14	15	15	14
Animals examined microscopically	15		1	15
Alimentary System				
None				
Cardiovascular System				
None				
Endocrine System				
None				
General Body System				
None				
Genital System				
None				
Hematopoietic System				
None				
Integumentary System				
Skin	(15)		(1)	(15)
Pinna, hemangiosarcoma			1 (100%)	
Subcutaneous tissue, fibrosarcoma				1 (7%)
Musculoskeletal System				
None				
Nervous System				
None				
Respiratory System				
None				

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

	0%	0.3%	1%	3%
Special Senses System				
None				
Urinary System				
None				
Neoplasm Summary				
Total animals with primary neoplasms[b]			1	1
Total primary neoplasms			1	1
Total animals with malignant neoplasms			1	1
Total malignant neoplasms			1	1

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] 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 Acesulfame Potassium: 0%

Number of Days on Study	190	274	274	274	274	274	274	274	274	274	274	274	274	274	274	274	
Carcass ID Number	15581	15512	15523	15534	15545	15556	15567	15579	15594	16060	16611	16612	16623	16644	16655		Total Tissues/ Tumors
Alimentary System																	
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
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
Mesentery									+								1
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		7
Pituitary gland	+	I	+	+	I	+	+	+	+	+	I	+	I	I	I		9
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
General Body System																	
None																	
Genital System																	
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Hematopoietic System																	
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Lymph node, mesenteric	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+		14
Lymph node, mediastinal									+								1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		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 Acesulfame Potassium: 0%

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

TABLE C2
Individual Animal Tumor Pathology of Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Acesulfame Potassium: 1%

Number of Days on Study	274	274	274	274	274	274	274	274	274	274	274	274	274	274	274	274	
Carcass ID Number	18101	18102	18103	18104	18105	18106	18107	18108	18109	18110	18111	18112	18113	18114	18115	18116	Total Tissues/ Tumors
Alimentary System																	
None																	
Cardiovascular System																	
None																	
Endocrine System																	
None																	
General Body System																	
None																	
Genital System																	
None																	
Hematopoietic System																	
None																	
Integumentary System																	
Skin														+			1
Pinna, hemangiosarcoma														X			1
Musculoskeletal System																	
None																	
Nervous System																	
None																	
Respiratory System																	
None																	
Special Senses System																	
None																	
Urinary System																	
None																	
Systemic Lesions																	
Multiple organs														+			1

TABLE C2
Individual Animal Tumor Pathology of Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Acesulfame Potassium: 3%

Number of Days on Study	225	274	274	274	274	274	274	274	274	274	274	274	274	274	274	
Carcass ID Number	19 99	19 96	19 97	19 98	20 00	20 01	20 02	20 03	20 04	20 05	20 06	20 07	20 08	20 09	21 00	Total Tissues/ Tumors
Alimentary System																
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
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
Mesentery		+														1
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	+	+	+	+	+	+	M	M	M	8
Pituitary gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	13
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
None																
Genital System																
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	I	+	I	13
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node															+	1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	14

TABLE C2
Individual Animal Tumor Pathology of Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Acesulfame Potassium: 3%

Number of Days on Study	225	274	274	274	274	274	274	274	274	274	274	274	274	274	274	
Carcass ID Number	19999	19967	19978	19988	20000	20001	20002	20003	20004	20005	20006	20007	20008	20009	20010	Total Tissues/ Tumors
Integumentary System																
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Subcutaneous tissue, fibrosarcoma	X															1
Musculoskeletal System																
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Nervous System																
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Respiratory System																
Larynx	+	+	+	+	M	I	+	M	M	M	I	I	+	+	I	7
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Special Senses System																
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Zymbal's gland	M	M	M	M	M	M	+	+	+	+	M	+	+	+	M	7
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

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

	0%	0.3%	1%	3%
All Organs: Malignant Neoplasms				
Overall rate[a]	0/15 (0%)	0/15 (0%)	1/15 (7%)	1/15 (7%)
Adjusted rate[b]	0.0%	0.0%	6.7%	6.7%
Terminal rate[c]	0/14 (0%)	0/15 (0%)	1/15 (7%)	0/14 (0%)
First incidence (days)	—[e]	—	274 (T)	225
Poly-3 test[d]	P=0.312	—[f]	P=0.509	P=0.509
All Organs: Benign or Malignant Neoplasms				
Overall rate	0/15 (0%)	0/15 (0%)	1/15 (7%)	1/15 (7%)
Adjusted rate	0.0%	0.0%	6.7%	6.7%
Terminal rate	0/14 (0%)	0/15 (0%)	1/15 (7%)	0/14 (0%)
First incidence (days)	—	—	274 (T)	225
Poly-3 test	P=0.312	—	P=0.509	P=0.509

(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.
[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 Acesulfame Potassium[a]

	0%	0.3%	1%	3%
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund	1			1
Survivors				
Terminal sacrifice	14	15	15	14
Animals examined microscopically	15		1	15
Alimentary System				
Liver	(15)			(15)
Inflammation, chronic active	1 (7%)			5 (33%)
Hepatocyte, inflammation, chronic active	1 (7%)			
Hepatocyte, necrosis, focal	1 (7%)			
Hepatocyte, centrilobular, hypertrophy				1 (7%)
Mesentery	(1)			(1)
Fat, inflammation, chronic active, focal	1 (100%)			
Fat, necrosis, focal				1 (100%)
Salivary glands	(15)			(15)
Infiltration cellular, focal, lymphocyte	10 (67%)			8 (53%)
Tongue	(15)			(15)
Inflammation	1 (7%)			
Cardiovascular System				
None				
Endocrine System				
Adrenal cortex	(15)			(15)
Hypertrophy, focal				1 (7%)
Capsule, inflammation, chronic active, diffuse	1 (7%)			
Subcapsular, hyperplasia, focal	1 (7%)			1 (7%)
General Body System				
None				
Genital System				
Epididymis	(15)			(15)
Infiltration cellular, lymphocyte	1 (7%)			
Polyarteritis				1 (7%)
Epithelium, degeneration, focal	1 (7%)			
Testes	(15)			(15)
Mineralization, focal	1 (7%)			
Sertoli cell, germinal epithelium, degeneration, 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 Acesulfame Potassium

	0%	0.3%	1%	3%
Hematopoietic System				
Lymph node				(1)
Inguinal, hyperplasia				1 (100%)
Lymph node, mandibular	(15)			(15)
Hyperplasia				2 (13%)
Lymph node, mediastinal	(1)			
Edema	1 (100%)			
Inflammation, chronic active, diffuse	1 (100%)			
Spleen	(15)			(15)
Hematopoietic cell proliferation	1 (7%)			1 (7%)
Thymus	(15)			(14)
Atrophy	2 (13%)			2 (14%)
Atrophy, focal				1 (7%)
Integumentary System				
Skin	(15)		(1)	(15)
Subcutaneous tissue, inflammation, chronic active, focal	1 (7%)			
Musculoskeletal System				
None				
Nervous System				
Brain	(15)			(15)
Inflammation, chronic active	1 (7%)			
Respiratory System				
Lung	(15)			(15)
Alveolar epithelium, inflammation, chronic active, focal	1 (7%)			1 (7%)
Nose	(15)			(15)
Inflammation, chronic active	1 (7%)			
Inflammation, granulomatous	1 (7%)			
Special Senses System				
Harderian gland	(15)			(15)
Infiltration cellular, lymphocyte				1 (7%)
Inflammation, chronic active	4 (27%)			2 (13%)
Urinary System				
Kidney	(15)			(15)
Inflammation, chronic active	1 (7%)			
Renal tubule, dilatation, focal	1 (7%)			2 (13%)
Renal tubule, nephropathy	1 (7%)			

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

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

	0%	0.3%	1%	3%
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund	1		1	
Natural deaths		1		1
Survivors				
Terminal sacrifice	14	14	14	14
Animals examined microscopically	15	1	2	15
Alimentary System				
Mesentery		(1)		
Sarcoma		1 (100%)		
Cardiovascular System				
Heart	(15)			(15)
Endocrine System				
None				
General Body System				
Tissue NOS	(1)			
Pelvic, osteosarcoma, metastatic, bone	1 (100%)			
Genital System				
None				
Hematopoietic System				
Lymph node				(1)
Pancreatic, histiocytic sarcoma				1 (100%)
Spleen	(15)			(14)
Histiocytic sarcoma				1 (7%)
Thymus	(15)			(14)
Histiocytic sarcoma				1 (7%)
Integumentary System				
Mammary gland	(15)		(1)	(13)
Osteosarcoma			1 (100%)	
Musculoskeletal System				
Bone	(15)			(14)
Femur, osteosarcoma	1 (7%)			

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

	0%	0.3%	1%	3%
Nervous System				
None				
Respiratory System				
Lung	(15)			(15)
Osteosarcoma, metastatic, bone	1 (7%)			
Special Senses System				
None				
Urinary System				
None				
Systemic Lesions				
Multiple organs[b]	(15)	(1)	(2)	(15)
Histiocytic sarcoma				1 (7%)
Lymphoma malignant				1 (7%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	1	1	1	2
Total primary neoplasms	1	1	1	2
Total animals with malignant neoplasms	1	1	1	2
Total malignant neoplasms	1	1	1	2
Total animals with metastatic neoplasms	1			
Total metastatic neoplasms	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 D2
Individual Animal Tumor Pathology of Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Acesulfame Potassium: 0%

Number of Days on Study	1 6 6	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	
Carcass ID Number	2 1 7	2 1 1	2 1 2	2 1 3	2 1 4	2 1 5	2 1 6	2 1 8	2 1 9	2 2 0	2 2 1	2 2 2	2 2 3	2 2 4	2 2 5		Total Tissues/ Tumors
Alimentary System																	
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
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	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+		14
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Parathyroid gland	+	+	M	+	+	+	M	M	M	M	+	M	M	+	M		7
Pituitary gland	+	+	+	+	I	+	+	M	+	+	+	+	+	+	+		13
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
General Body System																	
Tissue NOS	+																1
Pelvic, osteosarcoma, metastatic, bone	X																1
Genital System																	
Clitoral gland	I	+	+	+	+	M	M	M	+	M	M	+	M	+	M		7
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Vagina	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Hematopoietic System																	
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		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 Acesulfame Potassium: 0%

Number of Days on Study	1 6 6	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	2 7 4	
Carcass ID Number	2 1 7	2 1 1	2 1 2	2 1 3	2 1 4	2 1 5	2 1 6	2 1 8	2 1 9	2 2 0	2 2 1	2 2 2	2 2 3	2 2 4	2 2 5	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	M	+	M	+	M	+	+	+	+	I	+	+	+	+	+	11
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Osteosarcoma, metastatic, bone	X															1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Trachea	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	14
Special Senses System																
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Zymbal's gland	+	+	M	+	M	+	+	M	+	M	+	M	M	+	M	8
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	14
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE D2
Individual Animal Tumor Pathology of Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Acesulfame Potassium: 0.3%

Number of Days on Study	1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 6 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	
Carcass ID Number	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 2 3 3 3 3 3 3 3 3 3 3 4 7 6 7 8 9 0 1 2 3 4 5 6 8 9 0	Total Tissues/ Tumors
Alimentary System		
Mesentery	+	1
Sarcoma	X	1
Cardiovascular System		
None		
Endocrine System		
None		
General Body System		
None		
Genital System		
None		
Hematopoietic System		
None		
Integumentary System		
None		
Musculoskeletal System		
None		
Nervous System		
None		
Respiratory System		
None		
Special Senses System		
None		
Urinary System		
None		
Systemic Lesions		
Multiple organs	+	1

TABLE D2
Individual Animal Tumor Pathology of Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Acesulfame Potassium: 1%

Number of Days on Study	1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	
Carcass ID Number	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 4 4 4 4 4 4 4 4 4 5 5 5 5 5 5 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5	Total Tissues/ Tumors
Alimentary System		
None		
Cardiovascular System		
None		
Endocrine System		
None		
General Body System		
None		
Genital System		
Uterus	+	1
Hematopoietic System		
None		
Integumentary System		
Mammary gland	+	1
Osteosarcoma	X	1
Musculoskeletal System		
None		
Nervous System		
None		
Respiratory System		
None		
Special Senses System		
None		
Urinary System		
None		
Systemic Lesions		
Multiple organs	+ +	2

TABLE D2
Individual Animal Tumor Pathology of Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Acesulfame Potassium: 3%

Number of Days on Study	243	274	274	274	274	274	274	274	274	274	274	274	274	274	274	
Carcass ID Number	2557	2556	2558	2559	2560	2561	2562	2563	2564	2565	2566	2567	2568	2569	2570	Total Tissues/ Tumors
Alimentary System																
Esophagus	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Gallbladder	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
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	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Pancreas	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Salivary glands	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Stomach, forestomach	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Tongue	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Cardiovascular System																
Blood vessel	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Adrenal medulla	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Islets, pancreatic	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Parathyroid gland	A	+	M	M	+	M	+	+	+	+	+	M	+	M	+	9
Pituitary gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Thyroid gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
General Body System																
None																
Genital System																
Clitoral gland	A	+	+	+	+	M	M	+	M	M	+	+	+	M	+	9
Ovary	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Uterus	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Vagina	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Hematopoietic System																
Bone marrow	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node										+						1
Pancreatic, histiocytic sarcoma										X						1
Lymph node, mandibular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mesenteric	A	+	+	+	+	+	+	+	+	M	+	+	+	+	+	13
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Histiocytic sarcoma										X						1
Thymus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Histiocytic sarcoma										X						1

TABLE D2
Individual Animal Tumor Pathology of Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Acesulfame Potassium: 3%

Number of Days on Study	243	274	274	274	274	274	274	274	274	274	274	274	274	274	274	274	
Carcass ID Number	2557	2556	2558	2559	2560	2561	2562	2563	2564	2565	2566	2567	2568	2569	2570		Total Tissues/ Tumors
Integumentary System																	
Mammary gland	A	M	+	+	+	+	+	+	+	+	+	+	+	+	+		13
Skin	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Musculoskeletal System																	
Bone	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Skeletal muscle	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Nervous System																	
Brain	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Respiratory System																	
Larynx	A	+	+	+	+	+	+	+	+	I	+	+	I	I	+		11
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Nose	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Trachea	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Special Senses System																	
Eye	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Harderian gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Zymbal's gland	A	+	+	+	+	+	+	+	+	+	+	+	+	M	M		12
Urinary System																	
Kidney	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Urinary bladder	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Systemic Lesions																	
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Histiocytic sarcoma										X							1
Lymphoma malignant	X																1

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

	0%	0.3%	1%	3%
All Organs: Malignant Neoplasms				
Overall rate[a]	1/15 (7%)	1/15 (7%)	1/15 (7%)	2/15 (13%)
Adjusted rate[b]	6.7%	6.7%	6.7%	13.3%
Terminal rate[c]	0/14 (0%)	0/14 (0%)	0/14 (0%)	1/14 (7%)
First incidence (days)	166	176	127	243
Poly-3 test[d]	P=0.360	P=0.760	P=0.760	P=0.500
All Organs: Benign or Malignant Neoplasms				
Overall rate	1/15 (7%)	1/15 (7%)	1/15 (7%)	2/15 (13%)
Adjusted rate	6.7%	6.7%	6.7%	13.3%
Terminal rate	0/14 (0%)	0/14 (0%)	0/14 (0%)	1/14 (7%)
First incidence (days)	166	176	127	243
Poly-3 test	P=0.360	P=0.760	P=0.760	P=0.500

[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.

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

	0%	0.3%	1%	3%
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund	1		1	
Natural deaths		1		1
Survivors				
Terminal sacrifice	14	14	14	14
Animals examined microscopically	15	1	2	15
Alimentary System				
Liver	(15)			(14)
Infiltration cellular, focal, lymphocyte	2 (13%)			1 (7%)
Inflammation, chronic active	6 (40%)			7 (50%)
Hepatocyte, necrosis, focal	1 (7%)			
Salivary glands	(15)			(14)
Infiltration cellular, focal, lymphocyte	10 (67%)			9 (64%)
Cardiovascular System				
None				
Endocrine System				
Adrenal cortex	(15)			(14)
Hyperplasia, focal	2 (13%)			2 (14%)
Subcapsular, hyperplasia, focal	13 (87%)			12 (86%)
Thyroid gland	(15)			(14)
Ectopic thymus				1 (7%)
General Body System				
None				
Genital System				
Uterus	(15)		(1)	(14)
Endometrium, hyperplasia, cystic	13 (87%)		1 (100%)	13 (93%)
Hematopoietic System				
Lymph node, mandibular	(15)			(14)
Hyperplasia	1 (7%)			
Spleen	(15)			(14)
Hematopoietic cell proliferation	2 (13%)			1 (7%)
Thymus	(15)			(14)
Atrophy	6 (40%)			2 (14%)
Atrophy, diffuse	1 (7%)			
Atrophy, focal				4 (29%)

[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 Acesulfame Potassium

	0%	0.3%	1%	3%
Integumentary System				
Mammary gland	(15)		(1)	(13)
Inflammation, acute	1 (7%)			
Musculoskeletal System				
None				
Nervous System				
None				
Respiratory System				
Lung	(15)			(15)
Infiltration cellular, lymphocyte	1 (7%)			1 (7%)
Special Senses System				
Harderian gland	(15)			(14)
Inflammation, chronic active	1 (7%)			
Urinary System				
Kidney	(15)			(14)
Inflammation, chronic active				1 (7%)
Renal tubule, dilatation, focal	6 (40%)			2 (14%)
Renal tubule, nephropathy	1 (7%)			
Urinary bladder	(14)			(14)
Infiltration cellular, focal, lymphocyte				1 (7%)

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

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 13 Tg.AC hemizygous and 15 p53 haploinsufficient mice per exposure group; 1,000 total erythrocytes were counted to determine the percent polychromatic erythrocytes (PCEs).

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over exposure groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each exposure 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 exposed group is less than or equal to 0.025 divided by the number of exposure groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Results of the 9-month studies were accepted without repeat tests, because additional test data could not be obtained. Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

EVALUATION PROTOCOL

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

RESULTS

Acesulfame potassium did not increase the frequency of micronucleated NCEs in peripheral blood of male or female Tg.AC hemizygous mice administered 0.3% to 3% of the chemical in feed for 9 months (Table E1). A similar study was conducted in p53 haploinsufficient mice, and in this study, a significant exposure-related increase in the frequency of micronucleated NCEs was noted in males but not females; micronucleus frequencies in the 1% and 3% male groups were significantly elevated over the control group (Table E2). There was no significant alteration in the percentage of PCEs among total erythrocytes in either Tg.AC hemizygous or p53 haploinsufficient mice.

TABLE E1
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Tg.AC Hemizygous Mice Following Administration of Acesulfame Potassium in Feed for 9 Months[a]

Dose (%)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	% PCE
Male				
NTP-2000 feed[d]	13	1.42 ± 0.19		3.4
0.3	11	1.59 ± 0.18	0.3180	3.4
1	13	1.62 ± 0.23	0.2867	2.9
3	13	1.58 ± 0.19	0.3252	3.4
		P=0.391[e]		
Female				
NTP-2000 feed	12	1.29 ± 0.16		3.4
0.3	12	1.13 ± 0.22	0.7004	3.1
1	12	1.17 ± 0.27	0.6520	3.5
3	12	1.58 ± 0.23	0.1995	3.3
		P=0.098		

[a] Study was performed at SITEK Laboratories, Inc. The detailed protocol is presented by MacGregor *et al*. (1990); NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte.
[b] Mean ± standard error
[c] Pairwise comparison with the controls, significant at P≤0.008 (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 E2
Frequency of Micronuclei in Peripheral Blood Erythrocytes of p53 Haploinsufficient Mice Following Administration of Acesulfame Potassium in Feed for 9 Months[a]

Dose (%)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	% PCE
Male				
NTP-2000 feed[d]	14	1.57 ± 0.22		2.98
0.3	15	2.10 ± 0.24	0.0691	3.25
1	15	2.57 ± 0.19	0.0043	3.30
3	14	2.79 ± 0.33	0.0010	3.36
		P=0.003[e]		
Female				
NTP-2000 feed	14	1.29 ± 0.16		3.44
0.3	14	1.71 ± 0.17	0.0950	3.29
1	14	1.75 ± 0.21	0.0791	3.27
3	14	1.50 ± 0.26	0.2483	3.24
		P=0.467		

[a] Study was performed at SITEK Laboratories, Inc. The detailed protocol is presented by MacGregor *et al*. (1990); NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte.
[b] Mean ± standard error
[c] Pairwise comparison with the controls, significant at P≤0.008 (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
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

Procurement and Characterization of Acesulfame Potassium

Acesulfame potassium was obtained from Riedel-deHaen (St. Louis, MO) via Research Triangle Institute (RTI) (Research Triangle Park, NC) in one lot (8415-76-02 RTI), which was used in the 9-month studies in Tg.AC hemizygous mice and p53 haploinsufficient mice. Identity and purity analyses were conducted by the analytical chemistry laboratory, RTI, and the study laboratory (BioReliance Corporation, Rockville, MD); the study laboratory also conducted stability analyses. Reports on analyses performed in support of the acesulfame potassium studies are on file at the National Institute of Environmental Health Sciences.

Lot 8415-76-02 RTI of the chemical, a white, odorless, crystalline powder, was identified as acesulfame potassium 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 acesulfame potassium. The infrared and proton nuclear magnetic resonance spectra are presented in Figures F1 and F2.

The purity of lot 8415-76-02 RTI was determined by the analytical chemistry laboratory using high-performance liquid chromatography (HPLC), using Waters Chromatography (Milford, MA), an Alltima C_{18} column, 25 cm × 3.2 mm (Alltech; Chicago, IL), a mobile phase of 0.0125 M potassium dihydrogen phosphate (pH3.5):acetonitrile (100:0 for 5 minutes; 100:0 to 90:10 in 25 minutes; 90:10 for 10 minutes), a flow rate of 1.0 mL/minute, with ultraviolet detection at 230 nm. The study laboratory confirmed purity by major peak comparisons versus a reference sample from the same lot (stored at –20° C) using HPLC with a variation in the mobile phase (potassium dihydrogen phosphate:acetonitrile; 95:5) and a Hewlett-Packard model 1100 (Palo Alto, CA) instrument.

For lot 8415-76-02 RTI, HPLC indicated one major peak and no impurities. The analytical chemistry laboratory determined the purity to be greater than 99%; the study laboratory determined the purity to be 101% relative to the reference standard. The overall purity of lot 8415-76-02 RTI was determined to be 99% or greater.

To ensure stability, the bulk chemical was stored at room temperature under a headspace of inert gas, protected from light, in polyethylene bags inside plastic pails. Stability of the bulk chemical was monitored during the study by the study laboratory using HPLC. No degradation of the bulk chemical was observed.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared once every 2 to 4 weeks by mixing acesulfame potassium with feed (Table F1). 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 5 minutes. Formulations were stored in doubled polyethylene bags at room temperature for up to 35 days.

Homogeneity studies of the 0.1% and 5% acesulfame potassium formulations and stability studies of the 0.1% formulations were performed by the analytical chemistry laboratory using HPLC with a variation in the mobile phase ratio (95:5). Homogeneity studies of the 0.3% and 3% acesulfame potassium formulations were performed by the study laboratory using HPLC and the RTI extraction method modified to centrifuge samples before analysis. Homogeneity was confirmed, and stability was confirmed for at least 35 days for dose formulations stored in amber glass bottles at –20° C, –5° C, and 25° C, and when exposed to light and air for 7 days.

Periodic analyses of the dose formulations of acesulfame potassium were conducted by the study laboratory using HPLC. Samples of the formulation were extracted with 50:50 water/methanol, shaken for 10 minutes, and then centrifuged; theophylline (2 mg/mL) was used as an internal standard. During the 9-month studies, the dose formulations were analyzed five times; all 15 of the dose formulations analyzed were within 10 % of the target concentrations (Table F2). Animal room samples of these dose formulations were also analyzed, and 12 of 15 animal room samples were within 10% of the target concentrations (Table F2).

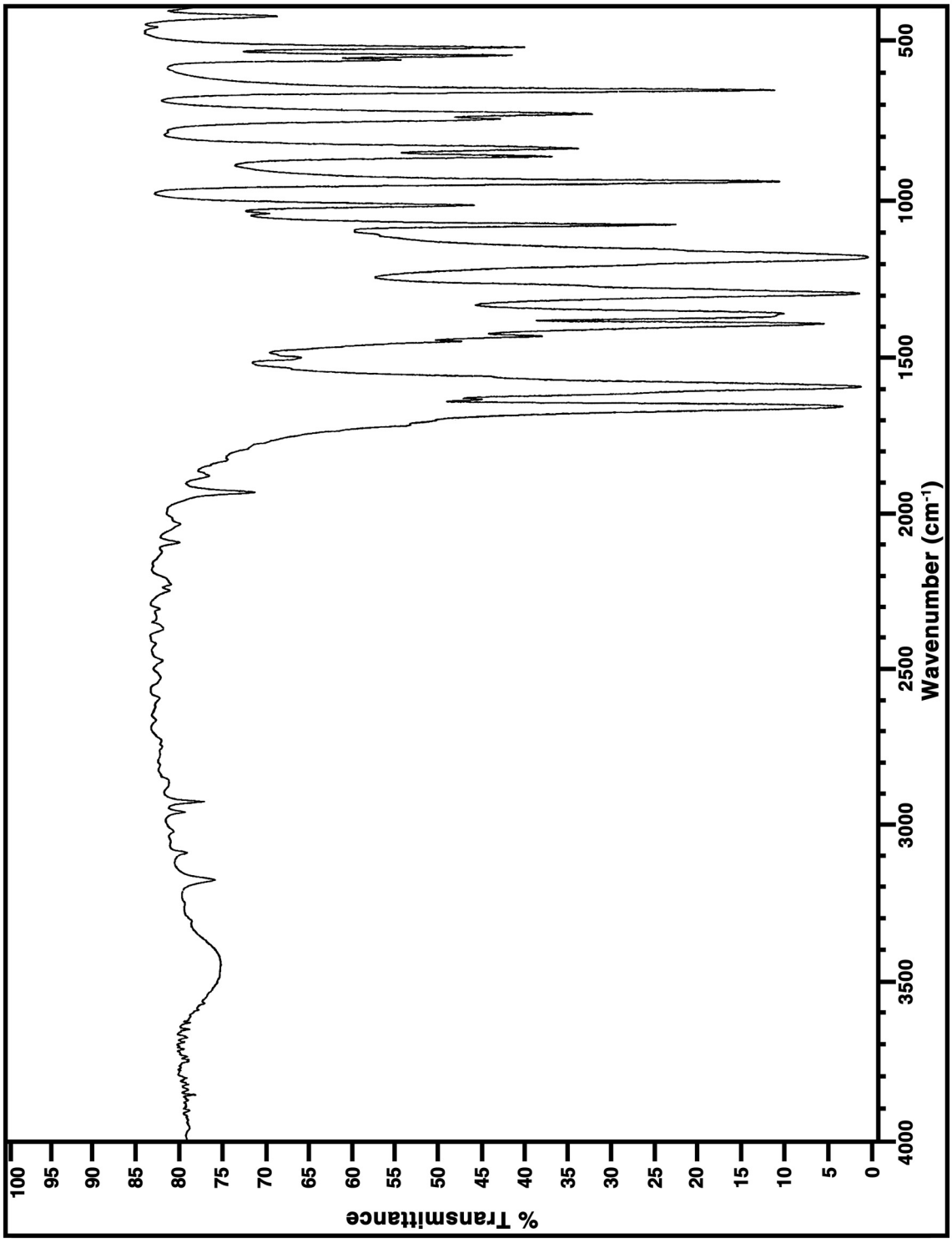

FIGURE F1
Infrared Absorption Spectrum of Acesulfame Potassium

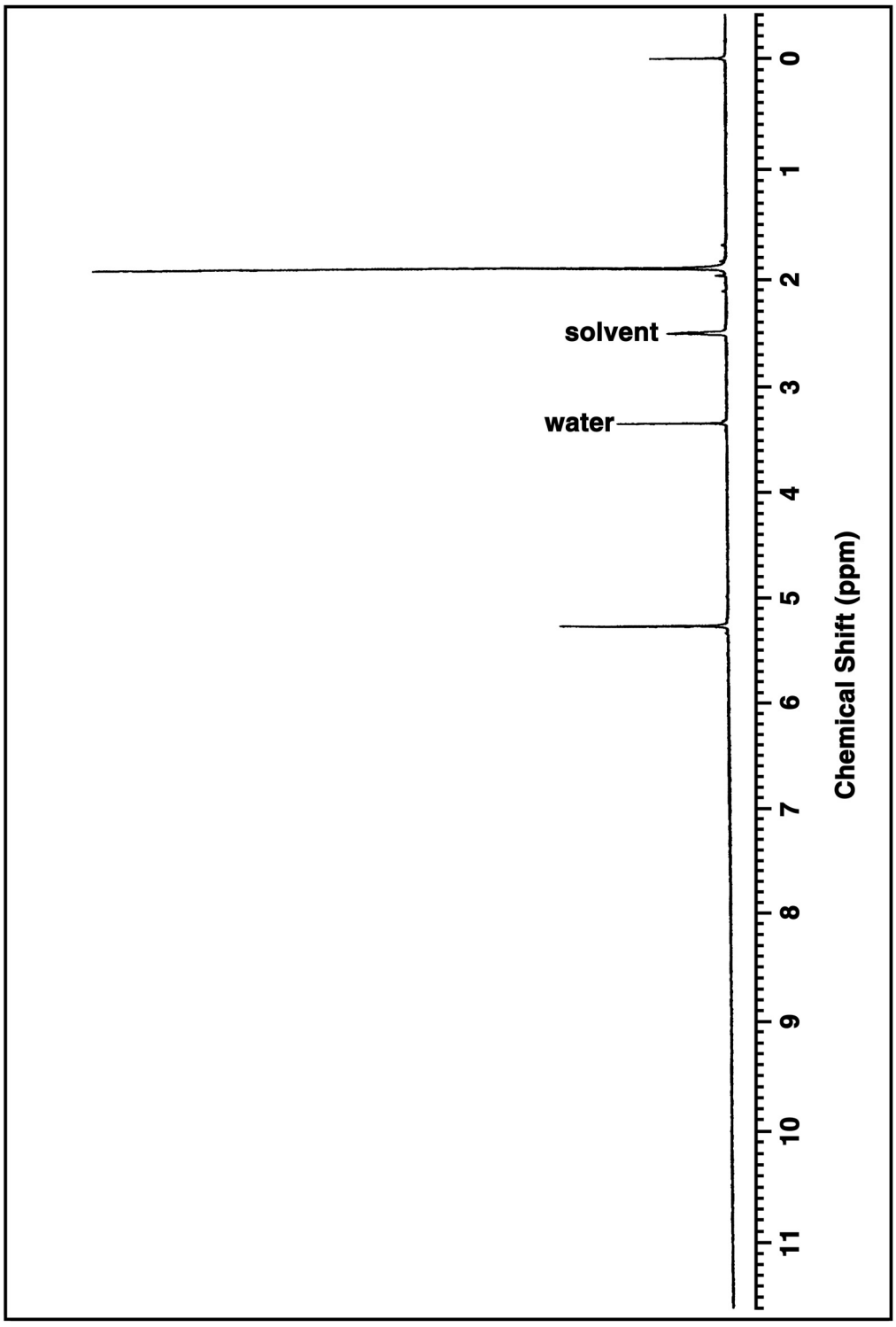

FIGURE F2
Proton Nuclear Magnetic Resonance Spectrum of Acesulfame Potassium

TABLE F1
Preparation and Storage of Dose Formulations in the 9-Month Feed Studies of Acesulfame Potassium

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

Chemical Lot Number
8415-76-02 RTI

Maximum Storage Time
35 days

Storage Conditions
Stored in doubled polyethylene bags at room temperature, protected from light

Study Laboratory
BioReliance Corporation(Rockville, MD)

TABLE F2
Results of Analyses of Dose Formulations Administered to Tg.AC Hemizygous Mice and p53 Haploinsufficient Mice in the 9-Month Feed Studies of Acesulfame Potassium

Date Prepared	Date Analyzed	Target Concentration (%)	Determined Concentration[a] (%)	Difference from Target (%)
January 19, 2000	January 19, 2000	0.3	0.283	–6
		1	1.06	+6
		3	2.89	–4
	March 7, 2000[b]	0.3	0.297	–1
		1	1.04	+4
		3	3.06	+2
February 29, 2000	February 29, 2000	0.3	0.305	+2
		1	1.02	+2
		3	3.14	+5
	April 19, 2000[b]	0.3	0.301	0
		1	0.857	–14
		3	3.01	0
May 9, 2000	May 9, 2000	0.3	0.298	–1
		1	0.985	–2
		3	3.01	0
	June 8, 2000[b]	0.3	0.295	–2
		1.	0.942	–6
		3.	3.04	+1
July 25, 2000	July 25, 2000	0.3	0.288	–4
		1	1.05	+5
		3	3.12	+4
	September 6, 2000[b]	0.3	0.293	–2
		1	0.847	–15
		3	2.76	–8
October 3, 2000	October 3, 2000	0.3	0.301	0
		1	1.01	+1
		3	3.08	+3
	November 8, 2000[b]	0.3	0.336	+12
		1	1.06	+6
		3	3.14	+5

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

APPENDIX G
FEED AND COMPOUND CONSUMPTION IN THE 9-MONTH FEED STUDIES OF ACESULFAME POTASSIUM

TABLE G1
Feed and Compound Consumption by Male Tg.AC Hemizygous Mice in the 9-Month Feed Study of Acesulfame Potassium

Week	0%		0.3%			1%			3%		
	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	22.6	4.1	22.0	554	4.5	22.7	1,978	4.6	22.5	6,093
3	4.2	23.3	4.4	23.6	564	4.5	23.7	1,901	4.6	23.7	5,838
4	4.3	24.2	4.3	24.9	524	4.5	24.8	1,817	4.6	24.7	5,615
5	4.2	24.9	4.4	25.7	509	4.3	25.3	1,686	4.4	25.1	5,309
6	4.2	25.5	4.1	26.3	470	4.4	26.4	1,659	4.5	25.6	5,290
7	4.1	26.3	4.1	26.5	460	4.3	26.8	1,607	4.5	26.9	5,021
8	3.8	25.9	4.2	27.2	467	4.3	27.5	1,567	4.5	27.4	4,918
9	4.2	27.4	4.1	27.6	445	4.1	27.7	1,464	4.3	27.7	4,656
10	4.1	27.5	4.1	27.6	450	4.2	28.0	1,515	4.4	27.7	4,739
11	4.1	27.0	4.2	28.2	445	4.3	28.4	1,528	4.4	27.6	4,790
12	4.2	28.0	4.3	29.0	444	4.4	29.2	1,502	4.6	28.8	4,832
13	4.0	28.2	4.2	28.9	436	4.3	29.4	1,463	4.5	29.0	4,683
14	3.9	28.8	4.1	29.8	413	4.2	29.7	1,418	4.5	29.3	4,589
15	4.1	29.3	4.3	29.9	434	4.4	30.0	1,458	4.6	29.9	4,575
16	4.0	29.5	4.0	29.8	406	4.2	30.3	1,402	4.5	30.1	4,485
17	4.2	29.6	4.2	29.8	420	4.4	30.5	1,454	4.7	30.4	4,669
18	4.1	29.2	4.4	30.5	429	4.4	30.7	1,440	4.5	30.6	4,399
19	4.2	29.4	4.0	30.4	396	4.1	30.5	1,331	4.5	30.7	4,399
20	3.9	29.5	4.0	29.7	409	4.2	30.5	1,391	4.4	30.6	4,342
21	4.0	29.5	4.2	30.1	417	4.3	31.2	1,367	4.4	31.1	4,232
22	3.8	29.5	4.1	30.9	393	4.3	31.6	1,359	4.6	31.5	4,379
23	4.1	30.4	4.1	32.0	387	4.1	31.4	1,298	4.4	31.8	4,130
24	4.1	31.5	3.9	31.1	374	4.0	31.8	1,242	4.4	31.9	4,156
25	4.0	31.6	4.4	32.2	408	4.2	32.2	1,299	4.4	32.1	4,073
26	4.0	31.8	4.2	32.2	393	4.1	32.2	1,264	4.5	32.8	4,109
27	4.0	32.0	4.1	32.2	386	4.2	32.2	1,318	4.4	32.3	4,065
28	3.8	32.0	4.2	32.6	386	4.2	32.9	1,276	4.4	32.8	4,037
29	4.3	32.4	4.3	32.8	394	4.2	33.1	1,280	4.3	32.5	3,977
30	3.9	32.5	4.2	33.3	378	4.1	33.4	1,231	4.3	33.0	3,948
31	4.1	32.5	4.2	33.3	377	4.2	33.1	1,266	4.2	33.0	3,778
32	4.1	32.5	4.2	33.2	379	4.3	33.6	1,287	4.7	33.7	4,187
33	4.1	32.3	4.1	32.5	379	4.2	33.2	1,280	4.3	33.0	3,888
34	4.1	32.2	4.4	32.7	402	4.3	33.4	1,281	4.4	33.4	3,935
35	4.3	32.6	4.2	32.8	383	4.5	33.4	1,346	4.3	33.4	3,818
36	4.3	33.0	4.3	32.7	390	4.5	33.7	1,325	4.5	33.4	4,071
37	4.4	32.7	4.3	33.5	387	4.6	34.1	1,344	4.6	33.3	4,171
38	4.3	32.9	4.6	33.5	412	4.4	33.4	1,318	4.6	33.2	4,162
39	4.2	33.2	4.0	32.9	361	4.4	34.4	1,275	4.6	33.9	4,070
40	4.2	33.2	4.5	33.6	400	4.2	34.1	1,245	4.5	33.5	4,059
Mean for weeks											
1-13	4.1	25.9	4.2	26.5	481	4.3	26.7	1,641	4.5	26.4	5,149
14-40	4.1	31.3	4.2	31.8	396	4.3	32.2	1,326	4.5	32.1	4,174

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

TABLE G2
Feed and Compound Consumption by Female Tg.AC Hemizygous Mice in the 9-Month Feed Study of Acesulfame Potassium

Week	0%		0.3%			1%			3%		
	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	3.6	17.5	4.3	18.2	710	3.9	18.2	2,160	4.5	18.1	7,467
3	4.3	17.8	4.1	18.5	660	4.3	18.3	2,339	4.0	18.3	6,630
4	4.5	19.6	4.1	19.5	625	4.1	19.3	2,106	4.1	19.7	6,299
5	4.3	19.7	3.8	19.6	582	3.8	19.8	1,944	3.9	19.7	5,992
6	4.0	19.7	4.1	19.5	626	3.8	19.6	1,948	4.1	19.4	6,303
7	3.9	20.3	3.9	20.1	588	4.2	20.5	2,029	4.1	20.6	5,926
8	4.0	20.7	4.1	20.8	592	4.0	21.2	1,902	4.0	20.8	5,771
9	3.9	21.3	4.0	21.6	559	4.0	21.5	1,875	4.0	21.2	5,672
10	4.0	21.7	3.9	21.6	548	4.1	21.9	1,880	4.2	21.4	5,897
11	4.2	22.1	4.0	21.9	546	4.4	22.2	1,960	4.4	22.3	5,953
12	4.2	22.5	4.2	22.1	573	4.3	22.6	1,892	4.2	21.9	5,801
13	4.1	22.2	4.0	22.4	538	3.9	22.7	1,737	4.2	21.9	5,770
14	3.8	22.3	4.0	22.7	533	3.9	22.7	1,719	4.0	22.1	5,411
15	4.3	22.5	4.0	23.1	514	4.1	22.9	1,785	3.9	22.6	5,230
16	4.2	23.5	4.0	23.3	512	4.0	23.2	1,705	4.2	23.4	5,448
17	4.0	23.7	4.2	23.6	528	4.2	23.2	1,828	4.1	23.0	5,316
18	4.1	24.0	3.8	23.4	491	4.1	23.7	1,738	4.2	23.3	5,408
19	4.0	23.8	3.9	23.3	500	3.9	23.9	1,615	4.1	23.3	5,249
20	4.3	24.4	4.0	23.5	507	4.2	23.9	1,760	4.3	23.7	5,447
21	4.1	24.6	4.0	23.8	498	4.3	24.4	1,781	3.7	23.5	4,754
22	4.0	24.9	3.9	24.0	488	4.0	24.6	1,628	3.9	23.3	5,026
23	4.3	25.0	3.8	24.1	478	3.7	24.2	1,544	4.5	24.0	5,614
24	4.1	25.4	4.0	24.5	491	4.1	24.8	1,641	4.1	24.7	4,996
25	3.8	25.3	4.0	24.5	487	3.8	24.7	1,527	4.0	25.1	4,827
26	4.1	25.4	4.0	24.8	488	4.2	25.0	1,668	4.2	25.2	4,973
27	4.3	26.7	4.2	25.3	499	3.9	25.2	1,545	4.0	25.1	4,840
28	4.1	26.5	3.8	25.5	448	3.8	25.1	1,525	4.1	25.1	4,893
29	3.9	26.8	3.9	25.2	467	4.0	25.1	1,597	3.9	24.8	4,672
30	3.8	27.1	4.0	25.6	465	3.8	25.4	1,483	4.1	25.7	4,758
31	3.9	26.7	4.0	25.5	465	3.9	25.5	1,536	4.1	26.1	4,755
32	4.0	26.4	4.1	25.7	477	4.3	26.0	1,641	4.3	26.1	4,934
33	3.9	25.9	4.2	25.8	482	3.8	25.5	1,502	4.1	25.8	4,701
34	4.1	26.0	4.0	26.1	465	3.8	25.4	1,493	4.2	25.6	4,882
35	4.0	26.0	3.9	24.9	470	4.1	25.9	1,586	4.4	26.4	5,048
36	4.6	28.3	4.3	25.9	493	4.1	26.3	1,573	4.4	26.2	5,021
37	4.5	28.4	4.3	26.4	487	4.3	26.6	1,615	4.2	26.3	4,813
38	4.3	27.8	4.2	26.7	475	4.0	26.6	1,508	4.5	26.6	5,042
39	4.6	28.5	4.1	26.6	460	4.0	26.8	1,491	4.2	26.7	4,763
40	4.4	28.5	4.5	26.3	515	4.4	27.0	1,639	4.3	26.6	4,851
Mean for weeks											
1-13	4.1	20.4	4.0	20.5	596	4.1	20.7	1,981	4.2	20.4	6,123
14-40	4.1	25.7	4.0	24.8	488	4.0	25.0	1,618	4.2	24.8	5,025

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

TABLE G3
Feed and Compound Consumption by Male p53 Haploinsufficient Mice in the 9-Month Feed Study of Acesulfame Potassium

Week	0%		0.3%			1%			3%		
	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	21.9	4.1	21.7	571	4.2	22.0	1,922	4.2	22.0	5,731
3	4.4	22.1	3.7	21.9	509	4.1	22.3	1,854	3.8	22.1	5,170
4	4.3	22.9	4.2	22.5	560	3.8	22.8	1,690	4.1	22.7	5,416
5	4.4	23.6	4.7	23.6	599	4.2	23.9	1,755	4.6	24.1	5,696
6	4.1	24.1	4.1	23.9	509	4.1	24.4	1,673	4.1	24.4	5,091
7	3.9	24.5	4.0	24.2	491	4.2	25.0	1,674	4.1	24.7	5,011
8	3.9	25.3	4.1	24.8	491	4.2	25.5	1,638	4.3	25.2	5,085
9	3.9	25.4	3.9	24.9	468	4.0	25.6	1,549	4.1	25.4	4,789
10	4.0	25.6	4.1	25.1	486	4.1	26.1	1,559	4.2	25.6	4,922
11	4.0	25.9	4.1	25.4	483	4.1	26.8	1,520	4.3	25.7	5,042
12	4.2	26.5	4.1	25.6	483	4.1	27.1	1,520	4.3	26.3	4,937
13	4.0	26.6	4.3	25.9	499	4.1	26.9	1,514	4.1	26.5	4,601
14	4.1	27.0	4.3	26.7	482	4.0	27.2	1,473	4.2	27.0	4,631
15	4.2	27.5	4.2	27.2	464	4.4	28.3	1,561	4.3	27.9	4,614
16	4.3	28.0	4.3	27.9	463	4.1	28.4	1,459	4.3	28.1	4,566
17	4.3	28.5	4.5	27.7	484	4.5	28.9	1,544	4.5	28.2	4,802
19	4.2	29.2	4.1	28.3	434	4.2	29.8	1,406	4.2	28.9	4,407
20	4.0	29.4	4.2	28.6	436	4.0	29.2	1,359	4.4	29.1	4,539
21	4.3	29.7	4.3	28.8	449	4.3	30.0	1,443	4.4	29.4	4,510
22	4.1	29.5	4.1	29.0	426	4.1	29.9	1,370	4.2	29.5	4,303
23	3.9	29.2	4.0	28.5	425	4.0	29.6	1,357	4.5	29.5	4,565
24	4.3	29.6	4.8	29.2	489	4.3	30.3	1,410	4.5	30.3	4,422
25	4.2	29.6	4.2	28.9	441	4.3	30.5	1,392	4.3	29.9	4,281
26	4.2	29.6	4.5	29.4	462	4.3	30.5	1,405	4.4	30.3	4,351
27	3.9	29.0	4.3	29.0	443	4.4	30.9	1,417	4.4	30.4	4,325
28	4.2	29.3	4.3	29.8	432	4.5	31.9	1,422	4.5	31.0	4,402
29	4.3	30.3	4.4	30.2	438	4.3	32.1	1,327	4.1	30.5	4,001
30	4.3	30.4	4.5	30.5	441	4.3	32.4	1,322	4.5	31.1	4,357
31	4.3	30.1	4.4	30.0	438	4.5	32.2	1,405	4.4	31.1	4,210
32	4.2	29.9	4.4	29.3	447	4.4	32.0	1,377	4.6	30.9	4,454
33	4.4	29.6	4.6	29.5	469	4.3	31.1	1,383	4.7	30.4	4,648
34	4.5	29.2	4.5	29.2	466	4.7	31.3	1,501	4.5	30.2	4,442
35	4.6	29.2	4.7	29.3	484	4.5	30.8	1,458	4.5	29.6	4,558
36	4.3	29.1	4.7	29.3	484	4.6	31.0	1,473	4.7	29.4	4,764
37	4.4	29.1	4.7	29.7	473	4.7	31.1	1,517	4.7	29.7	4,773
38	4.5	29.1	4.7	29.5	477	4.6	31.3	1,477	4.7	29.9	4,722
39	4.6	29.2	4.7	29.9	469	4.8	31.7	1,531	4.7	30.1	4,728
40	4.5	29.5	4.6	30.1	458	4.4	31.6	1,407	4.5	29.9	4,520
Mean for weeks											
1-13	4.1	24.5	4.1	24.1	513	4.1	24.9	1,656	4.2	24.6	5,124
14-40	4.3	29.2	4.4	29.1	457	4.4	30.5	1,431	4.4	29.7	4,496

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

TABLE G4
Feed and Compound Consumption by Female p53 Haploinsufficient Mice in the 9-Month Feed Study of Acesulfame Potassium

Week	0%		0.3%			1%			3%		
	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	18.5	4.2	17.9	708	3.7	18.4	2,027	4.0	17.8	6,756
3	4.4	18.4	4.5	18.7	719	4.2	19.2	2,207	4.3	18.5	6,987
4	4.3	19.6	4.4	19.5	674	4.3	19.9	2,160	4.0	19.1	6,327
5	5.0	20.2	5.1	20.7	737	4.7	20.6	2,282	4.8	20.1	7,184
6	4.5	20.9	4.8	21.1	685	4.5	21.2	2,124	4.7	21.0	6,771
7	4.2	21.0	4.6	21.2	645	4.0	21.5	1,871	4.0	21.1	5,757
8	4.7	21.4	4.3	21.5	595	3.8	21.5	1,789	3.9	20.8	5,654
9	4.5	21.4	4.2	21.5	587	3.9	21.7	1,801	4.1	21.3	5,844
10	4.4	21.8	4.4	21.6	608	3.9	21.9	1,797	4.2	21.6	5,798
11	4.4	22.4	4.6	22.4	616	4.2	22.4	1,888	4.3	21.8	5,890
12	4.7	22.6	4.8	22.3	644	4.3	22.6	1,895	4.7	22.3	6,271
13	4.4	23.0	4.9	23.1	633	4.6	23.2	1,995	4.5	22.8	5,940
14	4.5	23.2	4.5	23.6	574	4.6	23.5	1,937	4.5	22.9	5,873
15	4.4	23.6	4.9	24.2	605	4.4	23.7	1,862	4.2	22.9	5,543
16	4.9	24.1	4.9	25.2	578	4.6	24.2	1,906	4.8	23.6	6,146
17	4.7	24.3	4.8	25.1	578	4.6	24.2	1,919	4.7	23.7	5,980
18	5.1	24.7	5.1	25.6	601	4.8	25.0	1,912	4.7	24.1	5,874
19	4.9	24.8	4.6	25.8	531	4.7	24.9	1,888	4.8	24.6	5,855
20	4.9	25.1	4.6	26.1	526	4.5	25.5	1,784	4.8	24.9	5,816
21	5.0	25.4	4.8	26.4	549	4.4	25.7	1,720	4.7	25.3	5,575
22	4.6	25.6	4.6	26.8	517	4.5	26.1	1,708	4.7	25.5	5,483
23	4.8	26.1	5.0	27.0	550	4.4	26.3	1,682	5.0	25.6	5,878
24	4.3	26.1	4.7	26.9	522	4.5	26.6	1,679	4.6	26.0	5,317
25	4.6	26.4	4.6	26.9	516	4.4	26.7	1,647	4.5	26.3	5,190
26	4.6	26.5	4.8	27.1	535	4.6	26.5	1,721	4.9	26.3	5,568
27	4.6	26.7	4.4	27.3	479	4.5	27.2	1,657	4.8	26.9	5,391
28	4.7	26.9	4.6	27.8	495	4.4	27.4	1,596	4.7	27.4	5,167
29	4.5	27.0	4.4	28.0	475	4.5	28.0	1,618	4.7	27.1	5,150
30	4.7	28.2	4.8	28.6	501	4.4	28.5	1,548	5.0	28.0	5,357
31	4.7	27.8	4.9	28.6	512	4.7	28.5	1,634	4.7	27.9	5,020
32	4.7	28.1	4.9	27.9	531	4.6	28.5	1,622	4.7	27.5	5,127
33	4.7	27.5	5.1	28.0	546	4.9	28.4	1,718	5.3	27.9	5,736
34	4.6	27.7	5.1	27.8	552	4.6	28.3	1,641	4.8	27.5	5,274
35	4.8	27.6	5.3	27.3	581	4.7	27.7	1,690	4.7	26.8	5,284
36	4.4	27.3	4.8	28.1	510	4.7	28.2	1,668	4.6	27.1	5,049
37	4.8	27.5	5.0	28.0	539	4.7	28.0	1,676	4.7	27.6	5,054
38	4.6	27.7	4.5	28.1	483	4.8	27.9	1,708	4.8	27.6	5,267
39	4.5	27.5	4.9	28.3	521	4.6	28.2	1,617	4.9	28.0	5,213
40	4.7	28.3	4.9	28.8	514	4.8	28.8	1,677	4.7	28.3	4,968
Mean for weeks											
1-13	4.5	20.9	4.6	21.0	654	4.2	21.2	1,986	4.3	20.7	6,265
14-40	4.7	26.4	4.8	27.0	534	4.6	26.8	1,720	4.7	26.2	5,450

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

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