

Aspartame: Review of Safety

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DEDICATION

The authors dedicate this supplement to the memories of Lewis D. Stegink, Ph.D., and L. J. Filer, Jr., M.D., Ph.D., from the University of Iowa. Their early research on aspartame metabolism in humans formed the basis for much of the future research on aspartame that is discussed in this supplement. Their objectivity and long-standing dedication to science as well as their medical and scientific expertise are greatly missed.

PREFACE

More than 30 years have elapsed since the foundations of today's aspartame safety database were laid. Since that time the portfolio of studies assessing the safety of aspartame has continued to grow. A search of the scientific literature on the U.S. National Library of Medicine's MEDLINE reveals almost 700 citations for aspartame with a number of these relevant to aspartame safety. The extensive body of research undertaken on aspartame clearly and overwhelmingly demonstrates its safety for its intended use. The aspartame safety data have been evaluated and found satisfactory by regulatory scientists in all major regulatory agencies and expert committees, including the U.S. Food and Drug Administration (FDA), the EU Scientific Committee for Food (SCF), and the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Further, aspartame has been approved for human consumption by regulatory agencies in more than 100 countries and received wide consumer acceptance with consumption by hundreds of millions of people over the past 20 years, representing billions of man-years of safe exposure.

Thus, one would ask, "Why is there a need for a comprehensive review of aspartame safety data at this point in time?" Questions about its safety continue to be raised by a few individuals. Safety issues long ago resolved to the satisfaction of regulatory agencies and expert committees are today resurrected by some as if new. Early on these issues were based on scientific hypothesis, misinterpretation of data, and/or anecdotal reports of adverse health effects. Recently spurious and imaginative, hypothetical questions that are lacking even anecdotal support or a logical scientific rationale have compounded these issues. Given the extensive safety database and many years of safe human exposure, the continuing debate about aspartame is most unusual.

Those who have followed the "aspartame story" are familiar with the issues that continue to be raised.

- Aspartame is a simple molecule, which is hydrolyzed entirely to its constituent amino acids, aspartate and phenylalanine, and methanol which are then absorbed. The constituents of aspartame are also derived in much larger amounts from common foods. Are theoretical concerns for these components of any relevance to the small amounts that are produced following aspartame consumption under intended conditions of use?

- By what mechanism could the constituents of aspartame be handled differently in the body than are the chemically identical constituents from common foods? Since aspartame is broken down upon ingestion, what is the relevance of *in vitro* and parenteral studies that administer intact aspartame?

- Following the marketing of aspartame and the rapid and widespread consumer acceptance of aspartame-containing products, there were anecdotal reports of adverse health effects. Are these actually due to aspartame consumption? Why does the incidence wax and wane precisely when the media focus on these anecdotal reports?

- Critics have argued that aspartame induces brain tumors in rats. This conclusion was based originally on post hoc analyses of data combined from independent dose groups in animal carcinogenicity studies, but the conclusions reached by the critics were rejected by regulatory agencies. Thirty years later, the same critics again argued that aspartame induces brain tumors, on this occasion based on misinterpretation of epidemiology data; again this argument was considered in detail by regulatory bodies around the world and dismissed. How is the late-comer to distinguish credible concern from historical grievance?

To some extent, the current unwarranted concern about aspartame is fueled by the efforts of a small number of anti-aspartame activists who often seek to inflame as much as to inform. These individuals invest considerable personal time and energy in organizing and alerting others to what they believe to be the "dangers" of aspartame. Such individuals may be perceived by the public and the non-specialist media as representing an independent source of unbiased information, despite the fact that the conclusions they reach are not supported by the rigorous safety evaluations undertaken by respected regulatory agencies and expert committees or by publications in the peer-reviewed scientific literature.

One chain e-mail widely disseminated by lay anti-aspartame activists throughout the world purports to link aspartame with a long list of maladies including headache, joint pain, depression, memory loss, tinnitus, vertigo, blindness, carbohydrate cravings, Alzheimer's disease, birth defects, brain cancer, diabetes, Gulf War syndrome, lupus erythematosus, multiple sclerosis, and seizure disorders. Unfortunately, the scientific literacy of the public is sometimes insufficient to differentiate "Internet fiction" from "Internet fact." Well-meaning citizens repeatedly have brought these "findings"—perpetrated only on the Internet—to the attention of governments and regulatory agencies. Governments have to be responsive to such consumer concerns, despite the fact that continuing reevaluations are not warranted by the scientific evidence.

Misinformation about aspartame abounds elsewhere on the Internet, from websites such as www.aspartamekills.com to frenetic "news group" alerts flashed by anti-aspartame activists around the world. These inaccuracies have spilled over into the popular press, have affected governments and regulators, have been

published in scientific textbooks, have caused concern among practicing physicians, have required responses from organizations of health professionals, and have even affected research funding by charities. An Australian daily newspaper recently reported on “aspartame detox centers in America” as a serious news item. Today’s consumers frequently consult the Internet regarding a variety of health and nutritional issues; unfortunately, many users regard online information as authoritative and valid without questioning its source. Inappropriate advice on the Internet could pose a serious health danger to individuals needing medical treatment, because some activists recommend extended periods of aspartame abstinence in lieu of seeking medical attention for what could be symptoms of a serious and treatable illness. Whatever the goal of these individuals, it is not public health.

Considerable academic, regulatory, and industrial resources have gone into addressing issues raised regarding aspartame prior to its approval and during the many reaffirmations of its safety. As the present review will attest, considerable additional resources have been invested by many *after* the approval of aspartame. Readers should be aware that regulatory agencies have stringent premarket requirements for safety assessment; aspartame met and exceeded those requirements long ago and on numerous occasions since. However, the case of aspartame is unusual in that conclusions reached by regulatory agencies and authorities about the different issues are not accepted by some individuals, and the same allegations of adverse health consequences continue to be raised, despite their resolution to the satisfaction of competent authorities.

The present review considers all new published studies, puts recent reports into the context of previous experience, and discusses key aspects such as study design and methods, route of administration, and reproducibility. Anecdotal reports that aspartame has produced adverse health effects can only be considered by a rigorous evaluation of all the evidence. For example, in the case of epilepsy, given the prevalence of the condition and the millions of people who consume aspartame, it is inevitable that some individuals will experience their first seizure soon after consuming aspartame; such a temporal relationship is not evidence of causation, but it will appear convincing to the individual concerned. Such anecdotal experiences could be an indication of a cause–effect relationship, and therefore all those interested in ensuring public health, including the present reviewers, must consider the totality of the evidence that either supports or refutes such a relationship.

There is a new generation of scientists, regulators, and consumers who are factually unfamiliar with thirty years of aspartame research. The size and complexity of published literature documenting aspartame’s safety make issues and the resolution of questions difficult for those who have not followed the particulars from the beginning. Consequently, many today are ill-equipped to draw their own informed conclusions regarding the safety of aspartame. Thus, this comprehensive review of aspartame’s safety is both timely and warranted.

In this supplement, a group of esteemed scientists has accomplished the feat of compiling a comprehensive review of the extensive scientific literature published worldwide on aspartame safety. The reviewers have attempted to place more recent research into the continuum of earlier investigations. They have organized their reviews to address legitimate issues as well as theoretical concerns of critics.

The extensive list of contributing authors includes several scientists employed by the main producer of aspartame, and therefore activists may dismiss the findings here as biased. However, considering the credentials and bibliographies of all the contributing authors, and the detailed analysis of all relevant published data, it is clear that the reader can have confidence in using this review as an authoritative evaluation of the scientific literature. It is the intention of all the authors to present the scientific facts clearly and accurately so that the informed reader can decide whether there is a basis for continuing the debate on aspartame safety. Articles relevant to aspartame safety have been identified through ongoing searches of electronic databases such as Medline, Chemical Abstracts, Science Citation Index, Biosis, Food Science and Technology Abstracts, Agricola, and Toxline. The articles referenced in this review represent those identified from the 1970s into 2002.

The conclusion of this up-to-date review is in agreement with that of international regulatory agencies and expert committees: the weight of scientific evidence is clear that aspartame is safe for its intended use. The one exception is patients with the rare genetic disease, phenylketonuria, which is diagnosed at birth in the United States, Canada, Europe, and some other countries. These individuals are treated with low phenylalanine diets for a period of years, and while on this diet, they are required to monitor their phenylalanine intake from all dietary sources, including aspartame.

After 30 plus years of rigorous scientific research, it is time to put questions of aspartame safety to rest. It is difficult to identify any dietary constituent that has been more thoroughly evaluated than aspartame. The breadth and depth of scientific data available on aspartame and reviewed here are unlikely to exist for any other food additive. The continuing debate over such a “nonissue” only serves to divert attention and the allocation of resources from more important health issues that need to be addressed.

Introduction to Aspartame: Review of Safety

Over 20 years have elapsed since aspartame was approved by regulatory agencies as a sweetener and flavor enhancer. The safety of aspartame and its metabolic constituents was established through extensive toxicology studies in laboratory animals, using much greater doses than people could possibly consume. Its safety was further confirmed through studies in several human subpopulations, including healthy infants, children, adolescents, and adults; obese individuals; diabetics; lactating women; and individuals heterozygous (PKUH) for the genetic disease phenylketonuria (PKU) who have a decreased ability to metabolize the essential amino acid, phenylalanine. Several scientific issues continued to be raised after approval, largely as a concern for theoretical toxicity from its metabolic components—the amino acids, aspartate and phenylalanine, and methanol—even though dietary exposure to these components is much greater than from aspartame. Nonetheless, additional research, including evaluations of possible associations between aspartame and headaches, seizures, behavior, cognition, and mood as well as allergic-type reactions and use by potentially sensitive subpopulations, has continued after approval. These findings are reviewed here. The safety testing of aspartame has gone well beyond that required to evaluate the safety of a food additive. When all the research on aspartame, including evaluations in both the premarketing and postmarketing periods, is examined as a whole, it is clear that aspartame is safe, and there are no unresolved questions regarding its safety under conditions of intended use. © 2002 Elsevier Science (USA)

Since regulatory approval about 20 years ago, the high-intensity sweetener aspartame (L- α -aspartyl-L-phenylalanine methyl ester) is consumed in more than 6000 products by hundreds of millions of people around the world who want to enjoy the sweet taste of sugar without all the calories. The introduction of aspartame changed forever the quality of life of diabetic individuals by allowing them to enjoy good-tasting foods and beverages and still comply with dietary requirements. In addition, aspartame-containing products have been shown to be useful as part of a comprehensive multidisciplinary program to promote weight loss and control of body weight in obese individuals.

Aspartame is unique among high-intensity sweeteners because it is metabolized by digestive esterases and peptidases to three common dietary components—the amino acids, aspartic acid and phenylalanine, and a small amount of methanol. These components are used by the body in the same ways as when they are also derived from foods, such as meat, milk, fruits, and vegetables.

Further, the components of aspartame are derived from these common foods in much larger amounts. For example, a glass of no-fat milk provides about 6 times more phenylalanine and 13 times more aspartic acid and a glass of tomato juice provides about 6 times more methanol than an equivalent volume of beverage sweetened 100% with aspartame. Thus, much of the scientific research, both before and after regulatory approval, has focused on the safety of these components.

Prior to marketing, the safety of aspartame was firmly established through numerous studies using internationally accepted models for safety assessment. Extensive toxicologic and pharmacologic research was done in laboratory animals using much greater doses of aspartame than people could possibly consume. In addition, the safety of aspartame and its metabolic constituents was further confirmed in several human subpopulations: healthy infants, children, adolescents, and adults; obese individuals; diabetics; lactating women; and individuals heterozygous (PKUH) for the genetic disease phenylketonuria (PKU) who have a decreased ability to metabolize the essential amino acid phenylalanine. The results of the animal and human studies provided convincing evidence that aspartame consumption is safe, including by pregnant women and children. As a result, regulatory agencies in over 100 countries approved aspartame for its intended use as a sweetener.

In the postmarketing period, consumption studies demonstrated that actual aspartame intake at the 90th percentile was only 5–10% of the acceptable daily intake, thus providing small amounts of aspartate, phenylalanine, and methanol to the diet. Nonetheless, several scientific issues were raised and further evaluated in the postmarketing period, largely as a result of assertions of potential toxicity of its three metabolic components when given in extremely high doses. These issues centered on potential excitotoxicity of aspartic acid, potential effects of phenylalanine on brain function, and toxicity of methanol's formate metabolite. The results of the research demonstrated that it is not possible for a human to ingest enough aspartame to raise plasma concentrations of its metabolic constituents to those associated with adverse effects.

In response to anecdotal reports of adverse health effects from aspartame from some consumers, a postmarketing surveillance program was implemented to document and evaluate these reports. Although such reports cannot establish a cause-and-effect relationship, the information was used to guide additional research efforts, including evaluations of possible associations between aspartame and headaches, seizures, behavior, cognition, and mood, as well as allergic-type reactions. In addition, studies were done to evaluate safety of aspartame use by potentially sensitive subpopulations

(i.e., individuals heterozygous for the rare genetic disease phenylketonuria and individuals with Parkinson's disease, dizziness, depression, liver disease, and renal disease). Further evaluations of aspartame and the endocrine system and usefulness in weight control were completed. In addition, an allegation that aspartame may be associated with brain tumors in humans was raised by a long time aspartame critic almost 20 years after he first raised the same issue in animals. This allegation was evaluated by scientists and regulators with the conclusion that the claims were not valid.

The extensive literature included in this review represents over 30 years of research on aspartame safety. Articles relevant to aspartame safety were identified through ongoing searches of electronic databases such as Medline, Chemical Abstracts, Science Citation Index, Biosis, Food Science and Technology Abstracts, Agricola, and Toxline. Search terms included

aspartame, NutraSweet, aspartic acid, phenylalanine, methanol, and diketopiperazine. Research by scientists who have been involved with aspartame is also frequently monitored. For the most part, the referenced literature represents articles published in peer-reviewed journals; however, a few abstracts and book chapters were included when the information had not been published in peer-reviewed journals. The articles referenced in this review represent those identified from the 1970s into 2002.

The testing of aspartame has been far beyond the standard safety testing required to evaluate the safety of a food additive. When all the research on aspartame, including evaluations in both the premarketing and postmarketing periods, is examined as a whole, it is clear that aspartame is safe, and there are no unresolved questions regarding its safety under conditions of intended use.

Preclinical Safety Evaluation of Aspartame

Introduction

The original data used to support aspartame safety consisted of a comprehensive battery of studies in animals on acute, subchronic, and long-term toxicity, carcinogenicity, genetic toxicity, and reproductive toxicity and teratogenicity. In addition, there was a study to evaluate postnatal developmental effects in infant primates as well as studies of effects on the central nervous system, gastrointestinal tract, endocrine system, and reproductive system (Molinary, 1984; Kotsonis and Hjelle, 1996). The definitive preclinical studies done with aspartame are listed in Table 1. These studies demonstrated that aspartame is not toxic, carcinogenic, mutagenic, or teratogenic and has no effects on reproduction. Based on the results of these studies, a no-observed-effect level of at least 4000 mg/kg body wt was established by the Joint FAO/WHO Expert Committee on Food Additives (1980), the Scientific Committee for Food (SCF) (1985), and the Health Protection Branch of Health and Welfare Canada (1979). As a result, an acceptable daily intake (ADI) of 40 mg/kg body wt was set by those agencies based on the no-observed-effect level of 4000 mg/kg in the animal studies. The U.S. FDA

established a no-observed-effect level of 2000 mg/kg/day for the aspartame preclinical studies (FDA, 1974) based upon small changes in body weight in 4000 mg/kg body wt/day dose groups that other regulatory bodies accepted as being secondary to decreases in food consumption. The FDA (1974) set an ADI of 20 mg/kg body wt but subsequently raised it to 50 mg/kg body wt based on additional data from human studies (FDA, 1984).

In addition to aspartame, the safety of its conversion product, aspartylphenylalanine diketopiperazine (DKP), has been assessed in safety studies. DKP may form over time under conditions of extremes of pH and temperature, particularly in liquid matrices. The definitive preclinical safety studies done with DKP are listed in Table 2. JECFA established a no-observed-effect level of 750 mg/kg body wt/day for DKP from preclinical safety studies noting apparent findings in higher dose groups of one long-term rat study (JECFA, 1980). The U.S. FDA considered these observations incidental in older rats and established a no-observed-effect level of 3000 mg/kg body wt/day for DKP; FDA also evaluated additional data that became available after JECFA's deliberations (FDA, 1983). Thus, JECFA (1980) and the SCF (1985) set the ADI for DKP at 7.5 mg/kg body wt;

TABLE 1
Summary of Definitive Preclinical Toxicology Studies with Aspartame^a

Study type	Specific studies	Species	Outcome	Maximum dose (mg/kg body wt)
Genetic toxicology	Dominant lethal mutation assay	Rats	Negative	
	Host-mediated assay	Rats and mice	Negative	
	<i>In vivo</i> cytogenetics assay	Rats	Negative	
	Ames test	Standard strains	Negative	
Acute toxicology		Rats	No deaths	5000
		Mice	No deaths	5000
		Rabbits	No deaths	5000
Subchronic studies	Various lengths	Rats	Dose setting	
	Various lengths	Mice	Dose setting	
	Various lengths	Dogs	Dose setting	
Chronic toxicology and carcinogenicity	2 years	Rats	Negative	6000–8000
	2 years, <i>in utero</i> exposure	Rats	Negative	4000
	2 years (aspartame : DKP 3 : 1 Ratio)	Rats	Negative	3000 : 1000 aspartame : DKP ratio
	2 years	Mice	Negative	4000
	2 years	Dogs	Negative	4000
Reproduction	Two-generation	Rats	Negative	3500–7000
	Reproduction/fertility (segment I)	Rats	Negative	4100–4900
	Perinatal/postnatal (segment III)	Rats	Negative	4000–7000
	Postnatal (39 weeks)	Monkeys	Negative	3000
Teratology	<i>In utero</i> (segment II)	Rats	Negative	4100
	<i>In utero</i> (segment II)	Mice	Negative	5700
	<i>In utero</i> (segment II)	Rabbits	Negative	2400
	<i>In utero</i> (segment II) (aspartame : DKP 3 : 1 Ratio)	Rabbits	Negative	2000

^a Source: Kotsonis and Hjelle (1996).

TABLE 2
Summary of Definitive Preclinical Toxicology Studies with Aspartylphenylalanine Diketopiperazine (DKP)^a

Study type	Specific studies	Species	Outcome	Maximum dose (mg/kg body wt)
Genetic toxicology	Dominant lethal mutation assay	Rats	Negative	
	Host-mediated assay	Rats and mice	Negative	
	<i>In vivo</i> cytogenetics assay	Rats	Negative	
	Ames test	Standard strains	Negative	
Acute toxicology		Rats	No deaths	5000
		Mice	No deaths	5000
		Rabbits	No deaths	5000
Chronic toxicology and carcinogenicity	2 years	Rats	Negative	3000
	2 years (aspartame : DKP 3 : 1 ratio)	Rats	Negative	4000 DKP and 3000 : 1000 aspartame : DKP Ratio
	2 years	Mice	Negative	1000
Reproduction	Reproduction/fertility (segment I)	Rats	Negative	2000
	Two-generation (segment III)	Rats	Negative	2500
Teratology	<i>In utero</i> (segment II)	Rats	Negative	2000
	<i>In utero</i> (segment II) (aspartame : DKP 3 : 1 ratio)	Rats	Negative	3000 : 1000 aspartame : DKP Ratio
	<i>In utero</i> (segment II)	Rabbit	Negative	2000
	<i>In utero</i> (segment II) (aspartame : DKP 3 : 1 ratio)	Rabbit	Negative	3000 : 1000 aspartame : DKP Ratio

^a Source: Kotsonis and Hjelle (1996).

U.S. FDA (1983) set the ADI for DKP at 30 mg/kg body wt. Actual exposure to DKP in the United States at the mean and 90th percentile daily intakes have been estimated to be less than 0.25 and 0.56 mg/kg body wt/day, respectively (Kotsonis and Hjelle, 1996), well below the ADIs set by FDA, SCF, and JECFA.

It is not unusual for regulatory bodies to establish different no-observed-effect levels for the same preclinical safety data based on the different policies and practices of individual agencies. Consequently, regulatory bodies may apply different ADIs to compounds. For example, in the absence of toxicity, it is the normal practice of JECFA to recognize body weight changes due to reduced food consumption as not adverse when establishing a no-observed-effect level; the FDA generally considers these changes in body weight when establishing a no-observed-effect level. For example, in the case of sucralose, JECFA considered reduced body weight gain at 1500 mg/kg as being due to reduced palatability of diet and established an ADI of 0–15 mg/kg body wt/day based on a no-observed-effect level at this dose (JECFA, 1991). In contrast, FDA considered body weight changes and established a no-observed-effect level of 500 mg/kg body wt in rats and, consequently, an ADI for sucralose of 5 mg/kg body wt/day (FDA, 1998).

Notwithstanding the findings of national and international regulatory bodies that aspartame is not carcinogenic in animals, critics continue to cite a flawed and discredited analysis by Olney from the 1970s alleging that aspartame is associated with brain tumors in rats. Specifically, data from a long-term carcinogenicity study showed no dose–response relationship between

aspartame and brain tumors; however, in a post hoc analysis, data from independent dose groups were combined to imply a dose–response relationship. Specifically, data from the two different lowest dose groups were combined and contrasted with the combined data from the two different highest dose groups to allege an apparent dose response. Clearly, manipulations of data and dose groups in this manner are scientifically and statistically meaningless according to internationally accepted standards. Further, the assertion was made that another long-term bioassay in rats was invalid because the incidence of brain tumors in controls exceeded the evaluator's expectations. In actuality, no carcinogenicity study with aspartame or DKP (Tables 1 and 2) reveals a dose–response relationship between aspartame and brain tumors. Regardless of these long-lived claims, scientists in regulatory bodies and expert committees around the world, including the U.S. FDA (1981), the Canadian Health Protection Branch (Health and Welfare Canada, 1981), the UK Committee on Toxicity (MAFF, 1982), the EU Scientific Committee for Food (1985) and the Joint FAO/WHO Expert Committee on Food Additives (1980), which have evaluated the preclinical carcinogenicity data according to standard and internationally accepted criteria, have concluded that aspartame is not a carcinogen. For a complete discussion of the brain tumor issue see Evaluation of Aspartame and Brain Tumors below.

The large body of preclinical safety data that has been reviewed by regulatory bodies worldwide and establishes the safety of aspartame has been summarized elsewhere (Molinary, 1984; Ishii, 1984; Kotsonis

and Hjelle, 1996). Several studies in additional models have since become available which further expand the toxicology database for aspartame. These studies discussed below include evaluations of genetic toxicity, teratogenicity, and developmental milestones and behavioral development.

Additional Preclinical Safety Evaluations with Aspartame

Genetic toxicology. Tests done before regulatory approval demonstrated that aspartame was not genotoxic in the dominant lethal mutation assay in rats, the host-mediated assay in rats and mice, the *in vivo* cytogenetics assay in rats, and the Ames test (Kotsonis and Hjelle, 1996). Several additional studies have since been reported which further confirm the results of the original studies that aspartame is not genotoxic.

Karikas *et al.* (1998) measured direct molecular interaction of aspartame and its metabolites with DNA as an indicator of possible carcinogenic potential in an *in vitro* model. They observed measurable, dose-related molecular interaction with DNA by aspartame, as well as its amino acid components aspartic acid and phenylalanine. This was further verified in another article published by the same group (Schulpis *et al.*, 1998). What is being measured in this study is actually the interaction with DNA by amino groups characteristic of amino acids and is not an effect specific to aspartame. Further, aspartame is metabolized into its component amino acids and methanol which are then absorbed; i.e., aspartame itself is not absorbed (Ranney and Oppermann, 1979; Burgert *et al.*, 1991). Thus, aspartame is not available to interact with DNA *in vivo*. In another study, Jeffrey and Williams (2000) tested the DNA-damaging activity of several sweeteners in a rat hepatocyte/DNA repair assay. None of the sweeteners, including aspartame, showed an increase in NNG (net nuclear grain) counts; thus aspartame had no effect on DNA.

In an *in vitro* study, Kasamaki and Urasawa (1993) evaluated the ability of various food chemicals to induce cell aging in human diploid fibroblast cell cultures. Using known genotoxic materials (e.g., aflatoxin B₁), the cumulative cell population doubling (CPD) was reduced by 8–12 CPDs, whereas nongenotoxic compounds, such as aspartame, slightly shortened life span (2–6 CPDs) compared to untreated cells. However, the relevance of this research to human exposure is questionable, as aspartame is not absorbed into the systemic circulation.

Several studies have been done to evaluate potential clastogenic activity via chromosomal aberration evaluations. Durnev *et al.* (1995) gave daily aspartame doses of 40 and 400 mg/kg body wt by gavage over 5 days to mice. There was no clastogenic activity after aspartame. Kulakova *et al.* (1999) evaluated the effect of aspartame (0.4 to 40 mg/kg body wt) on the cytogenic effects of dioxydin and cyclophosphan in mice with chro-

mosomal aberration count in bone marrow cells. Aspartame alone appeared to have antimutagenic properties when injected 5 days before exposure to the mutagen, whereas joint administration of aspartame did not change clastogenic activity of the compounds. More recently, Mukhopadhyay *et al.* (2000) evaluated the effect of blends of aspartame (3.5, 35, 350 mg/kg body wt) and acesulfame-k (1.5, 15, 150 mg/kg body wt) by gavage in mice. There was no genotoxic effect as assessed by the presence of chromosomal aberrations in femoral bone marrow.

Teratogenicity. Bantle *et al.* (1990) evaluated five compounds, including aspartame, in the frog embryo teratogenesis assay (FETAX), a screening test developed as an alternative to mammalian teratogenesis. FETAX reportedly has a high degree of developmental relevance to mammals. For each compound, 8–16 concentrations were used in duplicate, and testing was done in a blinded manner except for one colored compound. The embryos were exposed to the compounds for 96 h. Malformations and embryo growth were evaluated. Based on the results of the study, the authors concluded that aspartame does “not pose a teratogenic hazard.” These results are consistent with those from the animal teratogenicity studies done preapproval with aspartame (Kotsonis and Hjelle, 1996).

Developmental milestones and behavioral development. Brunner *et al.* (1979) used pregnant rats to evaluate three doses of aspartame in the diet (2, 4, and 6%, equivalent to doses in pups of up to 3000, 6000, and 9000 mg/kg body wt/day) compared to a negative control as well as a positive control (hydroxyurea). In addition there was a group fed phenylalanine at 3% (up to 5000 mg/kg body wt/day) of the diet. Thus, pups were exposed from conception through weaning to 90 days of postnatal life. At aspartame doses to pups of 9000 mg/kg body wt/day or phenylalanine doses up to 5000 mg/kg body wt/day in the diet, deficits were seen in preweaning rats in time to eye opening, surface righting, locomotion (decreased), swimming, startle response, and open-field activity. Dramatically fewer effects were seen at the 6000 mg/kg body wt/day dose, and no effects were seen at the 3000 mg/kg body wt/day dose. Effects seen at the highest dose of aspartame were essentially the same as those observed with the phenylalanine dose. As reported in this study, pups received much higher doses than dams during the latter stages of lactation when pups began to eat solid food and consume the dams' aspartame-containing diets. For example, the young adult dams consuming 2, 4, and 6% aspartame in diet would be expected to be dosed with approximately 2000, 4000, and 6000 mg/kg body wt/day at these concentrations, respectively. Thus, the extremely high doses administered to pups during this study were sufficiently large to result in potential nutritional deficits in these

young, growing animals, which may account for any observed effects. These extremely large doses in pups are not relevant to human exposure to aspartame.

In a study comparing the effect of aspartame to saline-treated and untreated controls on development in mice, Mahalik and Gautieri (1984) administered doses by gavage to gravid mice on days 15–18 of gestation. Two doses of aspartame (1000 and 4000 mg/kg body wt) were evaluated. In the neonatal mice, there were no differences between either of the aspartame groups and historical controls in three of four parameters tested, including negative geotaxis or surface and air righting. However, using the rope method, achievement of the visual placing response was significantly delayed in both aspartame groups compared to historical control values and in the high-dose aspartame group compared to the low-dose group. Concurrent controls were not used. McAnulty *et al.* (1989) tested the same strain of mice with maternal dosages of 500, 1000, 2000, and 4000 mg/kg body wt aspartame by gavage on days 15–18 of gestation. In contrast to the findings of Mahalik and Gautieri, McAnulty *et al.* found no effect on the visual placing response when using concurrent controls. Specifically, there were no effects of any dose of aspartame on maternal body weight, food consumption, length of gestation, reproduction indices, or litter size. In the pups, birth weight, negative geotaxis, and surface and midair righting reflexes were not affected. Neither was there any delay in development of visual placing, regardless of the method used for assessment (grid or rope) or the manner in which data were analyzed after any dose of aspartame compared to concurrent controls. In addition, there were no differences between groups in eye opening, reflex pupil closure, and ophthalmologic examinations. Thus, the results of Mahalik and Gautieri were not reproducible.

Yirmiya *et al.* (1989) evaluated the effect of aspartame given in drinking water to dams starting 30 days prior to conception until rat pups were 30 days old. The average daily dose of aspartame in water was 1188 mg/kg body wt. Compared to rats in the plain water group, there were no differences in morphological development and reflexes in pups exposed to aspartame. At 30 days of age, there was a difference in performance in the radial arm maze in the aspartame-treated group. The authors observed that the number of entries prior to repeat were greater for aspartame-exposed rats than controls. They suggested that aspartame may either facilitate the development of components involved in this procedure or reduce factors that may interfere with this task. Holder (1989) reported another study to evaluate aspartame and development, including spatial memory in a radial arm maze. Adult female rats were exposed to aspartame or phenylalanine in drinking water for 12 days prior to mating until the pups were 38 days old. Doses of aspartame were 14, 68, 347, and 1614 mg/kg body wt/day, and phenylalanine dosing

averaged 835 mg/kg body wt/day. After weaning, pups consumed an average of 32, 154, 836, and 3566 mg/kg body wt/day aspartame, and those on phenylalanine averaged 1795 mg/kg body wt/day. No effects of aspartame were observed on reflex development or morphological development. In addition, contrary to the results of Yirmiya *et al.* (1989), there were no effects of aspartame compared to water on spatial memory as assessed in the radial arm maze. Evaluations included the number of arms chosen before one was reentered, overall time, and time in the arms. In addition, no differences were found between groups when pups were tested in the additional learning challenge of the milk maze.

In a testing paradigm in guinea pigs, Dow-Edwards *et al.* (1989) evaluated the effect of prenatal exposure (from conception to parturition) to aspartame (500 mg/kg body wt/day) in sesame oil compared to sesame oil alone and an untreated control group on odor-aversion testing. There were no significant effects on maternal weight gain, litter size, or birth weight of pups. At day 15 of age, pups were injected with lithium chloride (LiCl) or saline and exposed to vanilla odor for 30 min to create an aversion to vanilla odor. Twenty-four hours later, pups were permitted to choose between vanilla and lemon odors. The control and untreated groups showed the expected conditioned aversion to vanilla; however, pups in the aspartame group did not. The authors concluded that aspartame exposure throughout gestation disrupts odor-associative learning in 15-day-old guinea pigs. However, the validity of this model was questioned by a report from McAnulty *et al.* (1992), who did a feasibility study using sesame oil and untreated groups in anticipation of investigating the effects described by Dow-Edwards *et al.* (1989). They could not replicate either the conditioned odor aversion or the reported natural preference for vanilla over lemon odors in guinea pigs. As the group mean scores for vanilla preference were in the range of $50 \pm 15\%$ after saline or LiCl injections in either of the groups, there was no preference; pups sometimes showed preference for vanilla or lemon, but generally pups tended to remain in the first chamber entered regardless of the odor cue present. However, conditioned avoidance was readily demonstrated in an avoidance paradigm using electric shock, thus establishing that learning ability can be measured in 15-day-old guinea pigs. These results indicate that conditioned odor aversion in guinea pigs is not reproducible, and, thus, the methodology could not be used to investigate an odor aversion effect, such as that attributed to aspartame by Dow-Edwards and co-workers.

Conclusion

Since regulatory approvals of aspartame in the early 1980s, there have been a few additional animal safety studies completed to evaluate further the safety of

aspartame. In no cases have subsequent studies altered original determinations of safety established in the complete battery of preclinical studies evaluated by regulatory agencies as part of the approval process. One study suggested an effect of aspartame on DNA. However, other studies by the same investigators demonstrate the effect to be the result of an interaction with DNA by the amino group of amino acids in general and not an interaction specific to aspartame. Other investigators using another model assessing DNA damage found no effect of aspartame. One *in vitro* study suggested an effect of a number of food ingredients, including aspartame, on cell aging. Again, the lack of specificity of the response and the lack of applicability of these data to the *in vivo* situation eliminate the relevance of these data to the safety of aspartame's use in food. Several studies confirmed the results of earlier studies demonstrating the lack of genotoxicity of aspartame. Confirming the results of the preapproval safety studies, aspartame was also shown not to pose a risk of teratogenicity in an *in vitro* screening model.

In a study assessing developmental milestones and behavioral development after extremely large doses of aspartame, dose-related effects were seen but only at doses sufficiently large as to cause nutritional deficits. However, the same effects were also seen at an equivalent dose of phenylalanine demonstrating the effects were not specific to aspartame. Two studies appeared to indicate either an effect of aspartame on development of the visual placing response or a disruption of odor-associative learning. However, reported effects of aspartame on visual placing were not reproducible in an enhanced study using a concurrent control group by another laboratory, further the model of odor-associative learning also was not reproducible by others. In addition, reported effects of aspartame on performance in a radial arm maze were not reproducible.

In conclusion, an extensive body of preclinical safety data demonstrates the safety of aspartame and its hydrolytic cyclization product, DKP. This body of work demonstrates that aspartame and DKP do not produce adverse effects, even at doses several orders of magnitude greater than human consumption.

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Intake of Aspartame vs the Acceptable Daily Intake

Introduction

Prior to regulatory approval, extensive toxicologic and pharmacologic research was done in laboratory animals using much greater doses of aspartame than people could possibly consume. From the results of the toxicology studies, a no-observed-effect level of at least 4000 mg/kg body wt was established for aspartame by the Scientific Committee for Food (SCF) (1985), the Canadian Health Protection Branch (Health and Welfare Canada, 1979), and the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1980). Based on these assessments, an acceptable daily intake (ADI) of 40 mg/kg body wt was established for aspartame. When aspartame was first approved in the United States, the FDA established an ADI of 20 mg/kg body wt for aspartame (FDA, 1974).

In addition to the preapproval animal studies, the safety of aspartame and its metabolic constituents was assessed in humans in several subgroups: healthy infants, children, adolescents, and adults; obese individuals; diabetics; lactating women; and individuals heterozygous (PKUH) for the genetic disease phenylketonuria (PKU) who have a decreased ability to metabolize the essential amino acid, phenylalanine. The results of the human studies, along with the animal research, provided convincing evidence that aspartame was safe for general use, including by pregnant women and children. The FDA responded to these additional data by increasing the ADI for aspartame to 50 mg/kg body wt in 1983 (FDA, 1984). The ADIs for aspartame in the European Union and United States are the sweetness equivalent of a 60 kg person consuming approximately 500–600 g of sugar daily over a lifetime, obviously an amount well above consumption patterns for sugar.

As part of the safety evaluation for a food additive, regulators evaluate projected use levels relative to the ADI. If projected intake levels approach or exceed the ADI, restrictions may be imposed, such as limiting approvals for some categories of use to decrease potential exposure in the general population. Before approval, projected average intake levels of aspartame in the United States ranged from 8.3 mg/kg body wt/day if all sucrose in an average-sized person's diet was replaced by aspartame to 25 mg/kg body wt/day if all dietary carbohydrate could be replaced by aspartame. Based on dietary records from about 12,000 individuals, it was estimated that, if all possible foods were replaced with aspartame-containing foods, the 99th percentile daily consumption of aspartame would be 34 mg/kg body wt (FDA, 1981).

At the time of aspartame approval, FDA considered the 99th percentile estimated intake as representative of high-level consumers. However, since that time,

the FDA has concluded that the 99th percentile is unduly conservative and unreliable (FDA, 1986) because the very small number of consumers in the 99th percentile may have large and variable intakes from day to day, which may markedly skew the data. Thus, the FDA currently uses projections at the 90th percentile as the benchmark of high-level consumers. In the United Kingdom, the more conservative 97.5th percentile is used by regulators as representative of intake by high-level consumers (MAFF, 1990).

Aspartame Intake in the United States

Aspartame was approved by FDA for use in dry applications (e.g., tabletop, gelatins) in 1981 (FDA) followed by approval for use in carbonated soft drinks in 1983 (FDA). By 1984 about 30% of the U.S. population was estimated to consume aspartame, and MRCA Information Services (Northbrook, IL) began monitoring aspartame consumption in the U.S. population (Abrams, 1986, 1992; Butchko and Kotsonis, 1989, 1991, 1994, 1996; Butchko *et al.*, 1994, 2001; Butchko and Stargel, 2001, 2002). Intake was monitored from 1984 to 1992 via detailed menu census surveys from over 2000 households a year. During the 14-day survey, all foods eaten both at home and away from home were recorded.

Data were recorded by age group: 0–23 months, 2–5 years, 6–12 years, 13–17 years, and 18 years and over, as well as all age groups together. Intake by children was of special interest because of their smaller body weights as they may consume more of an additive on a milligram per kilogram basis than adults. In addition, intakes by special subpopulations, such as diabetics and people on weight-reduction programs, who might be enthusiastic users of aspartame with potentially higher intakes, and women of childbearing potential and pregnant women, were also monitored.

Because aspartame is so intensely sweet, only small amounts are needed to sweeten foods (Table 1). Thus, it would be expected that average daily intake of aspartame would be low. From the MRCA survey, the

TABLE 1
Approximate Aspartame Content of Some Common Foods and Beverages^a

Food	Serving size	Aspartame content (mg)
Beverage	12 fluid oz. (355 ml)	180
Yogurt	8 fluid oz. (240 ml)	125
Hot chocolate	6 fluid oz. (180 ml)	50
Tabletop sweetener	1 packet	35
Pudding dessert	4 fluid oz. (120 ml)	25

^a Source: Butchko and Stargel (2001).

TABLE 2

Aspartame Intake (mg/kg body wt/day) in the United States (90th Percentile, "Eaters" Only, 14-Day Average) from 1984 to 1992^a

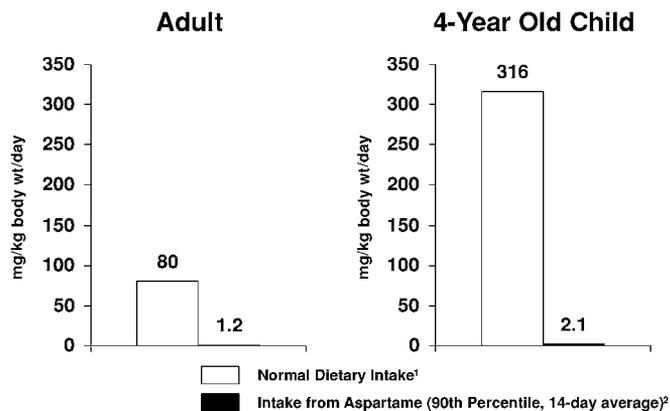
Dates of survey	General population	2–5 years	Individuals on a reducing diet	Women of childbearing age	Diabetics
1984–1985	1.6	3.1	1.6	2.0	2.1
1985–1986	2.1	4.8	2.2	2.2	2.2
1986–1987	2.1	3.7	2.3	2.5	3.0
1987–1988	2.3	2.6	2.6	2.8	3.3
1988–1989	2.2	4.0	2.5	2.6	2.6
1989–1990	2.5	3.1	2.7	3.2	2.7
1990–1991	2.8	3.5	2.8	3.7	3.4
1991–1992	3.0	5.2	3.3	4.2	3.3

^a Adapted from Butchko *et al.* (1994).

average daily intake over the 14-day period for the general population of aspartame "eaters" (at the 90th percentile) ranged from 1.6 to 3.0 mg/kg body wt/day from 1984 to 1992 (Table 2). Intake of aspartame at the 90th percentile, even by children, diabetics, people on weight-reduction diets, and females of childbearing age, was only about 5–10% of the ADI in the United States (50 mg/kg body wt).

Other types of consumption evaluations in the United States are consistent with the MRCA data. For example, 1-day diary data from the U.S. Department of Agriculture (USDA) Continuing Survey of Food Intakes by Individuals (CSFII) from over 1500 women showed that aspartame intake ranged from 0 to 16.6 mg/kg body wt/day with over 90% of the women who consumed aspartame reporting intakes less than 5 mg/kg body wt/day (Heybach and Smith, 1988). Although per capita disappearance data may underestimate consumption as both people who consume aspartame and those who do not are included, aspartame consumption for the total population (based on a 50-kg person) was estimated to be about 1.6 mg/kg/day based from USDA per capita disappearance data (Heybach and Allen, 1988).

Thomas-Doberson (1989, 1990) calculated potential aspartame intake by children from products, estimating how many servings of different products would need to be consumed to obtain intake at the ADI (50 mg/kg body wt/day in the United States) and at 34 and 17 mg/kg body wt/day. Unfortunately, some misinterpreted the article to conclude that children actually consume those large theoretical amounts of aspartame. In addition, the article was criticized because it raised various safety issues with incomplete citation of the scientific literature (Endres, 1990; Stenzel, 1990; Butchko, 1990). Subsequent data discussed above confirmed that, although one could devise a hypothetical diet to show that a child could consume large amounts of aspartame, actual intake by children is only about 10% of the ADI in the United States.



¹ From Stegink, 1984 (p. 512)

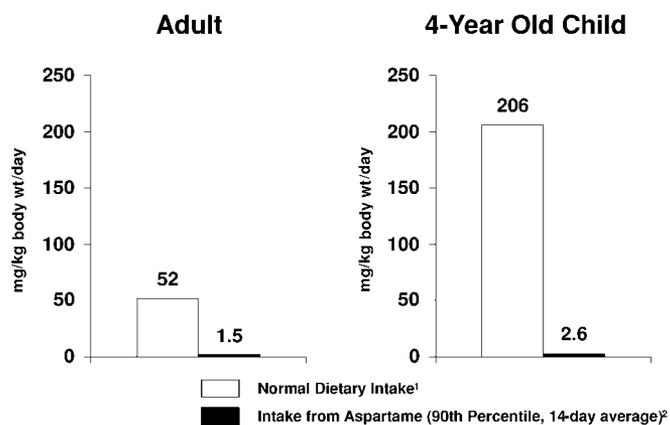
² From Butchko and Kotsonis, 1996

FIG. 1. Daily intake of aspartate from aspartame is small compared to typical daily dietary aspartate intake by adults and children. Adapted from Butchko and Kotsonis (1996).

From the intake data for aspartame, it is clear that aspartame (90th percentile intake) provides only small percentages of daily dietary intake of aspartate and phenylalanine for both adults and children (Figs. 1 and 2). In the case of adults, aspartame provides only about 2 and 3% of daily dietary intake of aspartate and phenylalanine, respectively. In the case of children, they consume much larger amounts of protein per kilogram of body weight for normal growth and development than do adults. Thus, aspartame provides only about 1% of daily dietary intake of both aspartate and phenylalanine for children.

Aspartame Intake in European Countries

Finland. Almost three-quarters of the diabetic children surveyed in Finland consumed aspartame-containing products. Mean intake of aspartame was



¹ From Stegink, 1984 (p. 512)

² From Butchko and Kotsonis, 1996

FIG. 2. Daily intake of phenylalanine from aspartame is small compared to typical daily dietary phenylalanine intake by adults and children. Adapted from Butchko and Kotsonis (1996).

1.15 mg/kg body wt/day, less than 3% of the ADI in Europe (40 mg/kg body wt) (Virtanen *et al.*, 1988).

France. Chambolle *et al.* (1994) reported that from 1991 to 1992, aspartame intake was 0.6 and 1.0 mg/kg body wt/day at the 90th and 95th percentiles, respectively. However, this study was limited because data for some categories were missing, and there were no data for food consumed outside the home. More recently, aspartame intake was evaluated in insulin-dependent diabetic children ranging in age from 2 to 20 years (Garnier-Sagne *et al.*, 2001) using a 5-day diary questionnaire. Intakes by aspartame consumers at the mean, 97.5th percentile, and maximum were 2.4, 7.8, and 15.6 mg/kg body wt/day, respectively. These results, however, are very conservative as all sugar-free products were assumed to contain only one sweetener at its maximum authorized level whereas many products actually contain blends of several sweeteners.

Germany. In a survey done in 1988–1989, consumption of the sweeteners, aspartame, cyclamate, and saccharin was evaluated in Germany. The 90th percentile average daily intake for aspartame was 2.75 mg/kg body wt/day (Bar and Biermann, 1992).

Italy. Italian teenagers who were known to be users of diet products had estimated average aspartame intakes of only 0.03 mg/kg body wt/day; the maximum aspartame intake was 0.39 mg/kg body wt/day (Leclercq *et al.*, 1999).

The Netherlands. Based on food frequency questionnaires, mean aspartame intake was estimated to be 2.4 mg/kg body wt/day. Intake at the 95th percentile intake was 7.5 mg/kg body wt/day. Using food intake records, mean and 95th percentile intakes were 1.9 and 7.5 mg/kg body wt/day, respectively (Hulshof and Bouman, 1995).

Norway. The average estimated intake of aspartame varied from 0.9 to 3.4 mg/kg body wt/day among males and females and various age groups (Bergsten, 1993).

United Kingdom. In a survey from 1988 in the United Kingdom, aspartame consumption at the 90th percentile was 4% of the ADI (40 mg/kg body wt) or about 1.6 mg/kg body wt/day. Children and diabetics ingested only 7 and 6%, respectively, of the ADI at the 90th percentile (Hinson and Nicol, 1992). From another survey done by the UK Ministry of Agriculture, Fisheries, and Food (MAFF, 1990), median and maximum aspartame intakes in 2- to 5-year-old children were 1.0 and 1.60 mg/kg body wt/day, respectively. In 35- to 64-year-old adults, mean and maximum aspartame intakes were 0.25 and 6.20 mg/kg body wt/day, respectively. In the general population, median, maximum, and 97.5th percentile intakes were 16, 372, and 109 mg, respectively, which are equal to 0.3, 6.2, and 1.8 mg/kg

body wt/day, respectively, for a 60-kg person. In 1994, the 97.5th percentile of aspartame consumption in diabetics, who would likely be frequent consumers of aspartame, was found to be 10.1 mg/kg body wt/day, only about 25% of the ADI (MAFF, 1995).

Aspartame Intake in Other Countries

Australia. Mean consumption levels of aspartame were 6 and 7% of the ADI (40 mg/kg body wt) for all respondents to a 7-day survey and total consumers, respectively. Although the 90th percentile consumption was 23% of the ADI, the small sample rendered a precise estimate of 90th percentile intake difficult (National Food Authority Australia, 1995).

Brazil. Aspartame intake at the median by the users of intense sweeteners was 2.9% of the ADI (40 mg/kg body wt); intakes at the median by diabetics and individuals on weight control regimens were 1.02 mg/kg body wt/day (2.6% of the ADI) and 1.28 mg/kg body wt/day (3.2% of the ADI), respectively (Toledo and Ioshi, 1995).

Canada. The general population of aspartame eaters in Canada consumed 5.5 mg/kg body wt/day during cold weather months and 5.9 mg/kg body wt/day during warm weather months (7-day average, 90th percentile) in 1987. Intake by children and special populations, who might consume higher amounts of aspartame, varied from 5.5 to 11.4 mg/kg body wt/day (eaters only, 90th percentile, 7-day average) (Heybach and Ross, 1989).

Conclusion

Dietary surveys with over 5000 individuals per year were done from 1984–1992 to monitor intake levels of aspartame in the United States. Average daily aspartame intake at the 90th percentile (eaters only) in the general population ranged from about 2 to 3 mg/kg body wt over the course of the surveys. Consumption by 2- to 5-year-old children in these surveys ranged from about 2.5 to 5 mg/kg body wt/day. Aspartame intake was also monitored in several other countries, including seven European countries. Although survey methodologies may have differed among these studies, aspartame intake is remarkably consistent across studies and is well below the ADI.

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Metabolism of Aspartame

Introduction

Aspartame (L- α -aspartyl-L-phenylalanine methyl ester) is metabolized in the GI tract into three components—the amino acids, aspartic acid and phenylalanine, and methanol (Fig. 1). Aspartame may be hydrolyzed into its components in the intestinal lumen followed by absorption of these components into the systemic circulation. At times, methanol is hydrolyzed in the intestinal lumen with transport of the aspartylphenylalanine dipeptide into mucosal cells, where it is metabolized to aspartate and phenylalanine, which are then absorbed into the systemic circulation. Aspartame may also be absorbed by intestinal mucosal cells where it is hydrolyzed to its components, which are then absorbed into the systemic circulation (Stegink 1984, 1987). Upon absorption, these constituents are metabolized, utilized, and/or excreted by the body in the same pathways as when they are derived from any other dietary source.

Common foods, such as milk, meat, fruits, and vegetables, actually provide far greater amounts of aspartic acid, phenylalanine, and methanol than does aspartame in the diet. Initially, there were concerns that the plasma concentrations of aspartame's constituents might be elevated above those known to be well tolerated. There were concerns expressed that aspartame may result in plasma concentrations of aspartate that may be associated with neurotoxicity (i.e., neuronal necrosis) (see Aspartate and Excitotoxicity below). It was also hypothesized that aspartame may markedly increase plasma phenylalanine concentrations and thereby affect brain function, which was a concern considering the adverse effects of elevated plasma phenylalanine concentrations associated with the rare genetic disease phenylketonuria (PKU) (see Phenylalanine and Neurochemistry below). In addition, as ingestion of large amounts of methanol, with consequent formation of formate, are associated with toxic

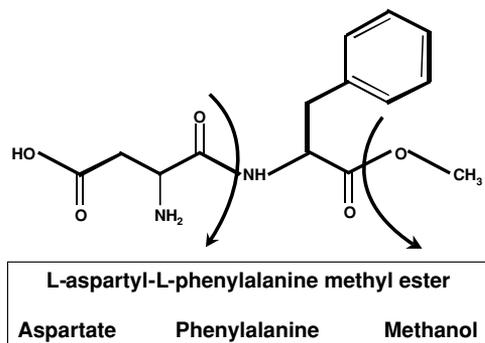


FIG. 1. Aspartame is metabolized by esterases and peptidases in the gastrointestinal tract into its three constituents—the amino acids, aspartate and phenylalanine, and methanol.

effects in sensitive species (e.g., optic nerve toxicity), the methanol derived from the methyl ester group of aspartame was studied (see Safety of Methanol from Aspartame and the Diet below). Thus, an extensive research program was undertaken before regulatory approvals to evaluate the plasma concentrations of aspartate, phenylalanine, and methanol after various doses of aspartame under different conditions. For the purpose of scientific completeness, additional studies were completed even after regulatory approvals were granted.

The metabolism of aspartame and the pharmacokinetics of its components have been studied in healthy adults, healthy infants, lactating females, adults heterozygous for phenylketonuria (PKUH), adults homozygous for phenylketonuria (PKU), and individuals thought to be sensitive to monosodium glutamate (MSG) ("Chinese restaurant syndrome"). Studies include acute bolus doses, repeated doses, administration in capsules to evaluate the appropriateness of capsules as a dosage form in blinded clinical studies, and aspartame ingested with meals, as well as long-term dosing studies. These safety studies have been extensively reviewed (Filer *et al.*, 1984; Filer and Stegink, 1988, 1989; Stegink, 1984, 1987; Stegink and Filer, 1996a,b). In addition, effects of aspartame on the ratio of phenylalanine (Phe) to the other large neutral amino acids (LNAAs) that compete for transport across the blood-brain barrier (Phe/LNAA) were evaluated (see Phenylalanine and Neurochemistry below).

Studies of Aspartame Metabolism

Extensive metabolism studies using radiolabeled aspartame were done in several animal species, including nonhuman primates, and humans to evaluate the metabolic fate of aspartame (Oppermann *et al.*, 1973; Ranney *et al.*, 1976; Ranney and Oppermann, 1979; Karim and Burns, 1996). The results of these studies demonstrated that aspartame is metabolized into its components in the GI tract; these are then absorbed and utilized by the body via the same metabolic pathways as when these same constituents are derived from common food.

Under conditions of extremes in pH, increased temperature, and long storage times, aspartame, like other aspartyl dipeptides, may cyclize to form the diketopiperazine cycloaspartylphenylalanine (DKP) and small amounts of β -aspartame. The safety of these conversion products of aspartame has been established (Kotsonis and Hjelle, 1996). DKPs are common components in food, e.g., protein-rich foods such as cocoa, cheese, protein, and casein hydrolysates, and in roasted malts used in brewing. Exposure to DKP from aspartame in products is limited because of product

acceptability issues due to loss of sweetness. Studies in animals and humans indicate that DKP is poorly absorbed, is not biotransformed by mammalian enzymes, and is rapidly eliminated in the urine. The β -aspartyl dipeptides are formed from the rearrangement of α -aspartyl dipeptides and may also be synthesized by kidney enzymes. At least 14 β -aspartyl dipeptides and 6 β -aspartyl tripeptides have been identified in human urine. β -Aspartylphenylalanine (β -AP), the free acid of β -aspartame, has also been isolated and identified in plasma and urine samples obtained from normal healthy subjects who had not consumed aspartame (see Kotsonis and Hjelle, 1996).

Burgert *et al.* (1991) evaluated the intestinal metabolism of aspartame at doses of 735 mg/kg body wt, phenylalanine methyl ester (PME) (450 mg/kg body wt), and phenylalanine (413 mg/kg body wt) administered to the proximal jejunum in pigs. The pig was chosen as an appropriate model for humans because of many structural and functional similarities with humans. Neither aspartame, nor phenylalanine methyl ester, nor aspartylphenylalanine (α -AP) was detected in portal blood, confirming the results of earlier studies that aspartame is not absorbed intact. This dose of aspartame is approximately 245 times 90th percentile average daily consumption of aspartame by the general population in the United States (Butchko and Kotsonis, 1991, 1996).

Stegink *et al.* (1995) evaluated α -aspartylphenylalanine hydroxylase activity and α -AP concentrations in preschool and school-age children (Wolraich *et al.*, 1994) who had received aspartame, sucrose, and saccharin for 3 weeks on each treatment. No α -AP was detected, and plasma phenylalanine and aspartate concentrations were within the normal range. Although one subject had α -aspartylphenylalanine hydroxylase activity well below normal, there were no behavioral or cognitive effects observed after aspartame.

Lipton *et al.* (1991) evaluated intestinal absorption of aspartame's metabolic components and conversion products in steady state perfusion studies of rat jejunum. The results demonstrated that α -AP and phenylalanine were rapidly taken up by a carrier-assisted system in the GI mucosal cells, but that β -AP and DKP may be absorbed through passive diffusion or excreted in the GI tract. Hooper *et al.* (1994) used microvillar membrane preparations from human duodenum, jejunum, and ileum and from pig duodenum and kidney to evaluate the metabolism of aspartame, α -AP, β -aspartame, and DKP by GI tract aminopeptidases. Expectedly, this study demonstrated that aspartame and α -AP were rapidly metabolized by aminopeptidases, with aminopeptidase A being the major activity in the microvillar membranes involved with the metabolism of aspartame. β -AP and DKP were essentially resistant to hydrolysis in human and pig microvillar membrane preparations.

In studies of the effect of aspartame on the activity of various liver xenobiotic-metabolizing enzymes, Tutelyan *et al.* (1989, 1990) administered aspartame (40 and 4000 mg/kg body wt) for 90 days to rats (these doses are about 13–1333 times 90th percentile average daily aspartame consumption in the United States) and concluded that aspartame, even in enormous doses, did not substantially affect hepatic microsomal enzyme function.

Studies in Healthy Adults and Infants

Single bolus dosing studies in healthy subjects. Studies using doses of aspartame ranging from those relevant to current consumption levels (4 and 10 mg/kg body wt), to doses representative of premarketing projections of high-level intake (34 mg/kg body wt) and the ADI in the United States (50 mg/kg body wt), to abusive doses of 100, 150, and 200 mg/kg body wt were undertaken (Table 1). The 200 mg/kg body wt dose approximated the amount of aspartame provided in about 25 liters of 100% aspartame-sweetened beverage consumed at one time by a 70-kg adult. Studies were also done in healthy 1-year-old infants given 34, 50, and 100 mg/kg body wt of aspartame.

In healthy adults, single bolus doses of aspartame administered at 4, 10, 34, and 50 mg/kg body wt (Stegink *et al.*, 1977, 1979a,b, 1987c; Wolf-Novak *et al.*, 1990) did not significantly affect plasma aspartate concentrations. Mean peak plasma aspartate concentrations in healthy adults administered abuse doses (100, 150, and 200 mg/kg body wt) of aspartame increased slightly after aspartame dosing (Stegink *et al.*, 1980, 1981a). However, all plasma aspartate concentrations (maximum approximately 1.0 μ mol/dl) were well within normal plasma aspartate concentrations observed postprandially in healthy human infants and adults (Stegink and Filer, 1996a). The results of the studies in infants given doses of aspartame of 34, 50, and

TABLE 1
Mean (\pm SD) Fasting Plasma Phenylalanine Concentrations, Mean Peak (\pm SD) Plasma Phenylalanine Concentrations, and Time to Mean Peak after Various Bolus Doses of Aspartame in Healthy Adults^a

Dosage (mg/kg body wt)	Fasting (μ mol/dl)	Mean peak (μ mol/dl)	Time to mean peak (min)
0	5.50 \pm 0.54	5.61 \pm 1.42	30
4	5.48 \pm 0.85	5.67 \pm 0.48	30
10	5.08 \pm 0.82	6.73 \pm 0.75	30
34	5.66 \pm 1.21	11.1 \pm 2.49	30
50	4.61 \pm 1.72	16.2 \pm 4.86	45
100	5.40 \pm 1.05	20.2 \pm 6.77	45
150	6.72 \pm 1.93	35.0 \pm 9.43	90
200	5.26 \pm 0.67	48.7 \pm 15.5	90

^a Adapted from Stegink *et al.* (1987c).

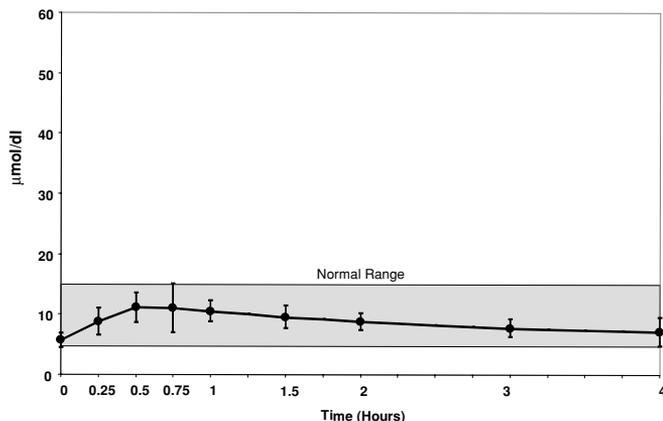


FIG. 2. Plasma phenylalanine concentrations (mean \pm SD) in healthy adults after a bolus dose of 34 mg/kg body wt aspartame remain within the normal postprandial range. Adapted from Stegink *et al.* (1979b).

100 mg/kg body wt demonstrated plasma aspartate concentrations similar to those observed in adults. Thus, aspartame was metabolized as well by infants as by adults (Filer *et al.*, 1983).

Plasma phenylalanine concentrations were not significantly increased in healthy adults after ingesting approximately 4 mg/kg body wt aspartame (Wolf-Novak *et al.*, 1990). A 10 mg/kg body wt bolus dose of aspartame increased plasma phenylalanine concentration from a mean (\pm SD) baseline concentration of 5.08 ± 0.82 $\mu\text{mol/dl}$ to a mean peak value of 6.73 ± 0.75 $\mu\text{mol/dl}$ 30 min after dosing (Stegink *et al.*, 1987c). Even after a 34 mg/kg bolus dose, mean peak plasma phenylalanine concentrations (11.1 ± 2.49 $\mu\text{mol/dl}$) were within the normal postprandial range (Fig. 2) (Stegink *et al.*, 1979b). After abuse doses of aspartame (100, 150, and 200 mg/kg body wt) (Stegink *et al.*, 1980, 1981a), mean peak plasma phenylalanine concentrations were approximately proportional to dose. Even after the highest dose (i.e., 200 mg/kg body wt), mean peak plasma phenylalanine concentrations (48.7 ± 15.5 $\mu\text{mol/dl}$) were well below those associated with adverse effects in untreated PKU (approximately 120 $\mu\text{mol/dl}$ or higher). Plasma phenylalanine concentrations in 1-year-old infants were similar to those noted in adults given the same doses (34, 50, and 100 mg/kg body wt) of aspartame. For example, the mean peak (\pm SD) plasma phenylalanine concentration after 34 mg/kg body wt aspartame was 9.37 ± 1.44 $\mu\text{mol/dl}$ in infants compared to 11.1 ± 2.49 $\mu\text{mol/dl}$ in adults.

Methanol metabolism was evaluated in healthy adult subjects administered aspartame at 34, 100, 150, and 200 mg/kg body wt (Stegink *et al.*, 1981c). After a dose of 34 mg/kg body wt aspartame, blood methanol concentrations remained below the limits of detection but increased significantly after doses of 100, 150, and 200 mg/kg body wt. The mean peak blood methanol concentrations increased in proportion to the dose. As

with aspartame's other constituents, metabolism in 1-year-old infants with aspartame doses of 34, 50, and 100 mg/kg body wt was similar to that in adults (Stegink *et al.*, 1983a).

The toxic effects of methanol in humans are due to the accumulation of its metabolite formate (Tephly and McMartin, 1984; Tephly, 1991; (see Safety of Methanol from Aspartame and the Diet). Blood and urine formate concentrations in subjects administered the highest dose of aspartame (200 mg/kg body wt) as a single bolus revealed no significant increase in blood formate concentration. Urinary formate excretion, however, increased significantly over baseline values in samples collected 0–4 and 4–8 h after aspartame ingestion. Thus, the rate of formate formation did not exceed the rate of formate excretion even after this enormous dose of aspartame (Stegink *et al.*, 1981c).

Multiple dose studies with aspartame in healthy subjects. In one study, 10 mg/kg body wt aspartame was administered every 2 h for three successive doses (Stegink *et al.*, 1988), which approximates the ingestion of about 4 liters of beverage sweetened with 100% aspartame by an adult in a 4-h period. There was no statistically significant effect on plasma aspartic acid concentrations, and only a small increase in plasma phenylalanine concentrations was noted. When a similar amount of aspartame (34 mg/kg body wt) was given as a bolus, the mean peak plasma phenylalanine concentration was 11.1 ± 2.49 $\mu\text{mol/dl}$ compared to 8.10 ± 1.42 $\mu\text{mol/dl}$ with the divided doses, demonstrating that mean peak plasma phenylalanine concentrations were lower when aspartame was administered in divided doses compared to an equivalent dose provided as a single bolus.

In the second study, 600 mg of aspartame was administered every hour for 8 h (Stegink *et al.*, 1989a), which approximates the amount of aspartame in about 10 liters of beverage sweetened with 100% aspartame given over 8 h. Aspartame had no significant effect on plasma aspartate concentrations. Plasma phenylalanine concentration increased from a mean baseline value of 5.45 ± 0.71 $\mu\text{mol/dl}$ to a mean peak value of 10.7 ± 1.35 $\mu\text{mol/dl}$ but remained within the normal postprandial range (Fig. 3). As expected, concentrations reached a steady state after about the fifth dose. These results indicated that a person could ingest over 1 liter of 100% aspartame-sweetened beverage every hour around the clock without increasing plasma phenylalanine concentrations beyond the normal postprandial range. Blood methanol concentrations were below the limit of detection after ingestion of this large amount of aspartame. There was no measurable effect on blood formate concentrations and no significant differences in urinary formate excretion after ingestion of aspartame or unsweetened beverage. Hence, the metabolism of methanol to formate and the renal clearance of

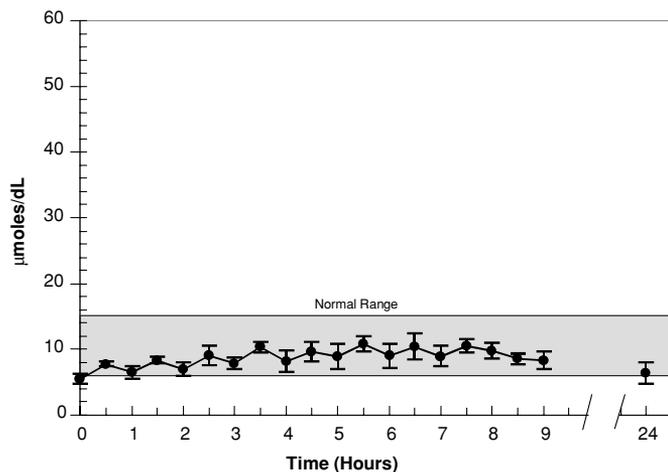


FIG. 3. Plasma phenylalanine concentrations (mean \pm SD) in healthy adults given 600 mg aspartame per hour for 8 h remain in the normal postprandial range. There is no accumulation of phenylalanine with repeated aspartame dosing. Adapted from Stegink *et al.* (1989a)

formate were sufficient to prevent methanol or formate accumulation in the body even after this large dose of aspartame (Stegink *et al.*, 1989a).

Long-term dosing with aspartame in healthy subjects. Before regulatory approvals of aspartame, “tolerance” studies using large doses of aspartame for up to 27 weeks were completed in healthy adults, children, and adolescents, obese subjects, individuals with diabetes, and individuals heterozygous for phenylketonuria (PKUH) (Frey, 1976; Knopp *et al.*, 1976; Koch *et al.*, 1976; Stern *et al.*, 1976). The results of these studies clearly demonstrated that plasma aspartate, phenylalanine, and methanol concentrations did not accumulate in the body following long-term exposures.

Subsequently, another comprehensive long-term exposure study using large doses of aspartame (Leon *et al.*, 1989) was completed in 108 healthy adult subjects. Subjects were given 75 mg/kg body wt aspartame or placebo daily in three divided doses for 6 months (i.e., the amount of aspartame in about 10 liters of beverage sweetened with 100% aspartame daily for an adult). In addition to extensive clinical and laboratory evaluations, measurements of plasma aspartate and phenylalanine concentrations, blood methanol and formate concentrations, and 24-hour urinary formate excretion were undertaken. There were no significant differences in plasma aspartate or phenylalanine concentrations between aspartame or placebo treatments. Likewise, there were no differences in Phe/LNAA. Most blood methanol concentrations were below the limit of detection in both the aspartame and placebo groups, and the number of subjects with detectable blood methanol concentrations were similar in both groups. Again, there was no accumulation of methanol from long-term in-

gestion of these very large doses of aspartame nor were blood formate concentrations significantly increased. Further, evaluation of 24-h urine collections revealed no increased excretion of urinary formate after aspartame administration compared to placebo or in urinary formate to creatinine ratio, indicating no significant increase in formate formation following high-dose, long-term aspartame intake.

Aspartame ingestion with meals in healthy subjects. The effect of aspartame ingested with meals (both high-protein and low-protein meals) on plasma amino acid concentrations has been evaluated in several studies (Stegink and Filer, 1996a). The high-protein meal used in the studies consisted of a hamburger/milk shake meal providing 1 g/kg body wt protein, containing 42 mg/kg body wt protein-bound phenylalanine (Stegink *et al.*, 1982, 1983b, 1991). After ingestion of the meal alone, plasma phenylalanine concentration increased from a fasting value of approximately 5 μ mol/dl to mean peak values ranging from 7.1 to 9.8 μ mol/dl. As noted previously, when 34 mg/kg body wt aspartame was administered as a single bolus, the mean peak plasma phenylalanine concentration was approximately 11.0 μ mol/dl (Stegink *et al.*, 1977, 1979b). However, when the same dose of aspartame was administered with the high-protein meal, the mean peak plasma phenylalanine concentration was 9.34 μ mol/dl. Plasma phenylalanine concentrations were not affected by a low-protein meal (0.005 g/kg body wt protein) consisting of consommé soup and unsweetened beverage (Stegink *et al.*, 1987a). However, when 34 mg/kg body wt aspartame was given with the low-protein meal, mean peak plasma phenylalanine concentration increased to 14.5 ± 4.53 μ mol/dl. The results of these studies indicate that ingestion of dietary proteins can modulate the aspartame-related increases in plasma phenylalanine concentrations.

Phe/LNAA after Aspartame Dosing in Healthy Subjects

All large neutral amino acids share a common transport mechanism across the blood–brain barrier, thus evaluation of the ratio of the plasma phenylalanine concentration to the sum of the plasma concentrations of the other large neutral amino acids (i.e., Phe/LNAA) would be more appropriate to evaluate entry of phenylalanine into the brain. As discussed in detail in the section Phenylalanine and Neurochemistry, it was suggested that simply evaluating the mean peak plasma phenylalanine concentrations after aspartame dosing may underestimate any potential effects of phenylalanine on the brain. It was further hypothesized that increased availability of phenylalanine to the brain and decreased availability of tyrosine could result in changes in brain neurotransmitter concentrations with consequent alterations in brain function.

A number of studies examined the effect of various doses of aspartame on Phe/LNAA. Expectedly, a high-protein meal provides phenylalanine in conjunction with other LNAAs and increased the plasma phenylalanine concentration but did not raise the Phe/LNAA above baseline (0.101 ± 0.010). However, ingestion of a single aspartame dose of 34 mg/kg body wt increased the Phe/LNAA from a baseline value of 0.102 ± 0.014 to a mean peak value of 0.230 ± 0.039 in healthy adults (Stegink and Filer, 1996a; Stegink *et al.*, 1979b). Plasma Phe/LNAA values were lower when approximately the same dose of aspartame was administered in divided doses to healthy adults. For example, ingestion of three successive 10 mg/kg body wt doses resulted in a mean peak Phe/LNAA of 0.161 ± 0.021 (Stegink *et al.*, 1988). When 34 mg/kg body wt of aspartame was added to a high-protein meal, the Phe/LNAA increased from 0.100 ± 0.017 at baseline to a mean peak of 0.130 ± 0.032 (Stegink and Filer, 1996a; Stegink *et al.*, 1982). Thus, ingestion of protein with aspartame, as would normally occur throughout the day, would reduce the change in the plasma Phe/LNAA related to aspartame.

Using a larger dose of aspartame (600 mg every hour for 8 h) in healthy adults revealed the mean peak Phe/LNAA of 0.22 ± 0.05 (Stegink *et al.*, 1989a). Phe/LNAA values as high as 0.125 ± 0.045 have been reported (Fernstrom *et al.*, 1979; Stegink and Filer, 1996a) in healthy adults under various dietary conditions, and a mean Phe/LNAA two standard deviations above this value would be 0.215. Thus, the mean peak Phe/LNAA observed when about 70 mg/kg body wt aspartame was given over 8 h was only slightly above the normal range despite the fact that the aspartame dose was well above current use levels (about 3 mg/kg body wt at the 90th percentile). In addition, the effect of aspartame on Phe/LNAA was amplified since subjects were fasted the entire day; thus, the normal ingestion of protein that would have modulated the increase in Phe/LNAA was absent.

The consumption of aspartame at levels more reflective of actual consumption (i.e., 4 mg/kg body wt) does not significantly affect the Phe/LNAA (Stegink *et al.*, 1987c). Further, such effects on Phe/LNAA are not unique to aspartame (see Phenylalanine and Neurochemistry below). Ingestion of carbohydrate, such as sugar, has similar effects on the Phe/LNAA (Martin-DuPan *et al.*, 1982; Burns *et al.*, 1991; Wolf-Novak *et al.*, 1990). Insulin-mediated uptake of large neutral amino acids (especially the branched-chain amino acids) after carbohydrate ingestion leads to decreased total plasma concentrations of the other large neutral amino acids relative to phenylalanine and thus an increase in the Phe/LNAA. Further, despite significant increases in Phe/LNAA after very large doses of aspartame, no consistent effects on brain function have been found in animal studies and controlled clinical studies (see Phenylalanine and Neurochemistry; Evaluation of As-

partame and Seizures and Electroencephalograms (EEGs); Evaluation of Aspartame and Headache; and Evaluation of Aspartame and Behavior, Cognitive Function, and Mood sections below).

Solution versus capsule administration in healthy subjects. In order to conduct randomized, double-blind, placebo-controlled studies to evaluate various issues raised regarding aspartame (e.g., headaches, seizures, allergic-type reactions) aspartame and placebo must be administered in a capsule form in order to mask aspartame's sweetness to maintain the integrity of the blinded condition. To evaluate whether there were pharmacokinetic differences in amino acid profiles after aspartame administered in solution compared to capsules, Stegink *et al.* (1987b) administered 3000 mg of aspartame (50 mg/kg body wt for a 60-kg adult) in solution on one occasion and in capsules on another occasion. Using this very large dose of aspartame, the mean peak plasma phenylalanine concentrations were higher and occurred earlier when aspartame was given in solution, and the 4-h area under the plasma phenylalanine curve (AUC) was greater after aspartame ingestion in solution compared to capsules. However, in blinded clinical studies, large doses are typically administered as divided doses. When lower and more relevant doses (20 mg/kg body wt) were used (Burns *et al.*, 1990), there were much smaller differences between plasma phenylalanine pharmacokinetics after capsules compared to solution. This dose is similar to those used in focused clinical studies. Thus, capsules provided an appropriate dosage form for blinded clinical studies.

Aging and aspartame metabolism. Puthrasingam *et al.* (1996) evaluated the metabolism of aspartame in elderly (65–80 years) subjects compared to young subjects. Subjects were given 40 mg/kg body wt aspartame, and pharmacokinetics (C_{\max} and AUC) of plasma phenylalanine and tyrosine concentrations were determined. There were significantly higher C_{\max} and AUC for phenylalanine in the elderly group compared to the young subjects; however, there were significant decreases in volume of distribution and clearance in the elderly, which accounted for these differences. The authors concluded that the differences were modest and were not indicative of a need to change the ADI for aspartame in the elderly.

Studies in Individuals Heterozygous for Phenylketonuria (PKUH)

As individuals with the rare, genetic disease phenylketonuria (PKU) cannot properly metabolize phenylalanine, there was interest in whether individuals heterozygous for PKU (carriers) would be able to metabolize the phenylalanine derived from aspartame so that plasma concentrations did not reach levels seen in untreated PKU (about $120 \mu\text{mol/dl}$ or higher). PKUH

have approximately one-half the normal liver capacity to metabolize phenylalanine; however, they are able to sufficiently metabolize phenylalanine so that fasting plasma phenylalanine concentrations are only slightly higher than those in healthy individuals. As a result, studies were done to evaluate the effect of bolus doses of aspartame, repeated doses of aspartame, and aspartame given with a protein meal in PKUH.

Single-bolus dosing studies in PKUH. After a dose of 10 mg/kg body wt aspartame in PKUH (Stegink *et al.*, 1987c; Stegink and Filer, 1996b), plasma phenylalanine concentrations increased from a mean baseline of $9.04 \pm 1.71 \mu\text{mol/dl}$ to a mean peak value of $12.1 \pm 2.08 \mu\text{mol/dl}$ at 30 min after aspartame dosing. In healthy subjects, this dose of aspartame increased plasma phenylalanine concentrations from 5.09 ± 0.820 to $6.73 \pm 0.750 \mu\text{mol/dl}$. As expected, plasma aspartate concentrations were similar in healthy and heterozygote subjects, while methanol levels were below the limits of detection. After a 34 mg/kg body wt dose of aspartame (Stegink *et al.*, 1979b), plasma phenylalanine concentrations increased significantly in both healthy subjects and PKUH, with concentrations in the PKUH being significantly higher. However, plasma phenylalanine concentrations in PKUH remained within the normal postprandial range. Even after a very large dose of aspartame (100 mg/kg body wt) (Stegink *et al.*, 1980), mean peak plasma phenylalanine concentrations in PKUH ($41.7 \pm 2.33 \mu\text{mol/dl}$) were well below those observed in untreated PKU. The 100 mg/kg body wt dose of aspartame is equivalent to the amount of aspartame in approximately 12 liters of beverage sweetened with 100% aspartame for a 60-kg individual consumed in a single sitting. It was evident that PKUH cleared phenylalanine at about half the rate of healthy individuals. Yet, even high intakes of phenylalanine from aspartame would be adequately metabolized by PKUH.

Repeated dosing studies in PKUH. Two multiple-dose studies were undertaken in PKUH—one study involved ingestion of aspartame at 10 mg/kg body wt every 2 h for three successive doses (Stegink *et al.*, 1989b), and the other study involved consumption of aspartame at 600 mg per serving every hour for 8 h (Stegink *et al.*, 1990). In the three-dose study, mean peak plasma phenylalanine concentrations were not significantly different than the normal postprandial range for PKUH. In addition, mean peak plasma phenylalanine concentrations after repeated doses were significantly lower than after a similar dose of aspartame when given as a bolus.

In the repeated-dose protocol with eight hourly doses of aspartame, plasma phenylalanine concentrations increased significantly and reached a steady state after five doses. Plasma phenylalanine concentrations were only slightly above the postprandial range for PKUH (Fig. 4) and were well below values associated with adverse effects in children with PKU. This study con-

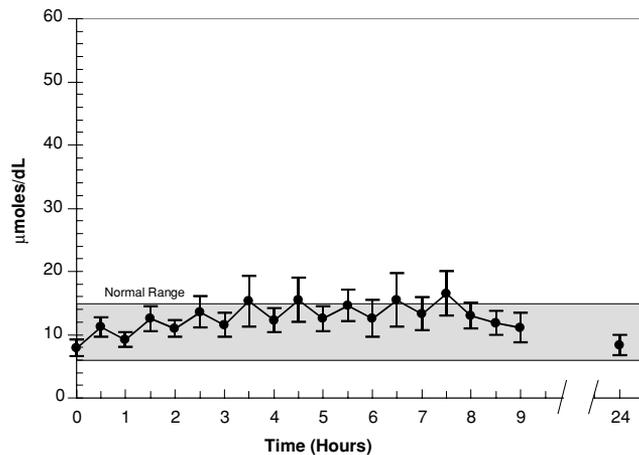


FIG. 4. Plasma phenylalanine concentrations (mean \pm SD) in PKU heterozygous adults given 600 mg aspartame per hour for 8 h approximate the normal postprandial range for this population. There is no accumulation of phenylalanine with repeated dosing. Adapted from Stegink *et al.* (1990).

firmed that a PKUH could ingest the equivalent of over 1 liter of beverage sweetened with 100% aspartame at hourly intervals around the clock without increasing plasma phenylalanine concentrations appreciably above the postprandial range.

Aspartame ingestion with meals in PKUH. The effect of aspartame (10 and 30 mg/kg body wt) plus a hamburger/milk shake meal on plasma phenylalanine concentrations in PKUH was evaluated (Stegink and Filer, 1996b). The test meal provided protein at 1 g/kg body wt (equivalent to 42 mg/kg body wt protein-bound phenylalanine) (Stegink *et al.*, 1991). After ingestion of the high-protein meal only, plasma phenylalanine concentrations increased from a fasting level of $7.72 \pm 1.13 \mu\text{mol/dl}$ to a mean peak value of $12.6 \pm 2.11 \mu\text{mol/dl}$. Only minimal additional increases in plasma phenylalanine concentrations were found with the addition of aspartame (10 mg/kg body wt) to the meal (Stegink and Filer, 1996b). When aspartame at 30 mg/kg body wt was added to the high protein meal, the mean peak plasma phenylalanine concentration was $17.0 \pm 4.24 \mu\text{mol/dl}$. Curtius *et al.* (1994) also found that addition of aspartame to a high-protein meal increased plasma phenylalanine concentrations in PKUH, but the mean peak was only slightly above the normal postprandial range for PKUH. Thus, as in healthy subjects, dietary protein can modulate the rise in plasma phenylalanine concentrations after aspartame in PKUH compared to concentrations that would be expected from the dose of aspartame administered alone.

Studies in Phenylketonuric Homozygotes (PKU)

PKU individuals on special low-phenylalanine diets are discouraged from ingesting aspartame-sweetened

products, which are labeled to inform these individuals that the product contains phenylalanine. However, questions have arisen regarding the safety of aspartame ingestion by PKU individuals no longer on a low-phenylalanine diet.

Caballero *et al.* (1986) evaluated plasma phenylalanine concentrations at baseline and 1 h after aspartame (10 mg/kg body wt) in healthy subjects, PKU heterozygotes, mild hyperphenylalaninemics, and PKU homozygotes not being treated with a low-phenylalanine diet. Baseline and post-dose plasma phenylalanine concentrations were 4.45 ± 1.29 vs 5.80 ± 0.95 $\mu\text{mol/dl}$ in healthy subjects; 6.88 ± 1.38 vs 8.23 ± 1.73 $\mu\text{mol/dl}$ in PKU heterozygotes; 41.2 ± 20.8 vs 41.3 ± 1.84 $\mu\text{mol/dl}$ in mild hyperphenylalaninemics; and 136.9 ± 24.0 vs 132.3 ± 21.0 $\mu\text{mol/dl}$ in PKU homozygotes.

Wolf-Novak *et al.* (1990) evaluated plasma phenylalanine concentrations after ingestion of a 355-ml serving of 100% aspartame-sweetened beverage (about 4 mg/kg body wt) in healthy subjects and adolescent PKU individuals who were no longer on a low-phenylalanine diet. The aspartame-sweetened beverage had no significant effect compared to baseline on plasma phenylalanine concentrations in healthy subjects (5.39 ± 0.470 $\mu\text{mol/dl}$) or in adolescent PKU subjects (150 ± 23 $\mu\text{mol/dl}$). Mackey and Berlin (1992) also evaluated the effect of aspartame in a 355 ml serving of beverage vs a sugar-sweetened beverage on plasma phenylalanine and tyrosine concentrations in healthy subjects and PKU individuals no longer on a phenylalanine-restricted diet. There were no significant increases in plasma phenylalanine and tyrosine concentrations in healthy subjects. Likewise, in PKU individuals, plasma phenylalanine and tyrosine concentrations did not differ significantly from baseline.

The results of these studies demonstrated that aspartame (equal to that in up to 1 liter of beverage sweetened with 100% aspartame consumed as a single bolus) does not increase plasma phenylalanine concentrations above the already very elevated levels in mild hyperphenylalaninemics and untreated PKU individuals.

Metabolism after Administration of Aspartame Plus Monosodium Glutamate (MSG)

The ingestion of large amounts of MSG may produce a variety of symptoms (e.g., burning sensation, facial pressure, and chest pain) in sensitive individuals, usually referred to as “Chinese restaurant syndrome.” Because of aspartame’s aspartate content and the structural similarity between glutamate and aspartate, concern about aspartame consumption along with MSG in foods suggested that aspartame might precipitate symptoms in subjects allegedly sensitive to MSG. Stegink *et al.* (1981b) gave MSG-sensitive subjects aspartame (34 mg/kg body wt) or sucrose (1 g/kg body wt) using a randomized, double-blind, crossover experimental design. No test subject reported symptoms typical of

an MSG-type response after consuming either sucrose or aspartame. Plasma aspartate concentrations were not significantly different after aspartame loading than after sucrose loading. The results of this study indicate that subjects who allegedly experienced idiosyncratic reactions to MSG did not experience similar symptoms after ingestion of either aspartame or sucrose.

In a randomized, crossover study, Stegink *et al.* (1982) evaluated the effect of aspartame (34 mg/kg body wt) plus MSG (34 mg/kg body wt) ingestion on plasma and erythrocyte amino acids concentrations in healthy adults fed a high-protein (1 g/kg body wt) meal compared to the meal alone. The addition of aspartame plus MSG had little effect on plasma or erythrocyte concentrations of either aspartate or glutamate beyond those from the meal alone. In another similar study (Stegink *et al.*, 1983b), subjects were given a high-protein (1 g/kg body wt) meal, a high-protein meal with 150 mg/kg body wt MSG, and a high-protein meal with 150 mg/kg body wt MSG plus 23 mg/kg body wt aspartame. Ingestion of MSG significantly elevated plasma glutamate plus aspartate concentrations above those from the meal alone. The addition of aspartame to the meal with MSG did not increase plasma glutamate plus aspartate concentrations above those after the meal with MSG.

Stegink *et al.* (1987a) also evaluated the effect of MSG and aspartame on plasma amino acid concentrations when given a low-protein meal. Study conditions included the meal alone, the meal plus MSG (50 mg/kg body wt), and the meal plus MSG (50 mg/kg body wt) and aspartame (34 mg/kg body wt). Plasma glutamate or aspartate or glutamate plus aspartate concentrations were not affected by the meal alone but were significantly increased after MSG and MSG plus aspartame conditions. The AUC for plasma aspartate plus glutamate concentrations, however, did not differ between the MSG compared to MSG plus aspartame conditions. The results of these studies demonstrated that symptoms of “Chinese restaurant syndrome” elicited by MSG were not elicited by aspartame. In addition, the results do not support the contention that aspartame consumed with MSG results in rapid and dangerous rises in plasma aspartate and glutamate concentrations.

Conclusions

Numerous studies have evaluated the metabolism of aspartame and the pharmacokinetics of its components— aspartate, phenylalanine, and methanol. Such studies have involved healthy adults, infants, children, and adolescents, PKU heterozygous adults, PKU homozygous individuals, and individuals sensitive to MSG. Studies included acute-dose, repeated-dose, and long-term dose regimens. In healthy adults and children, even after enormous doses, aspartame does not result in plasma concentrations of its components that are of safety concern. Plasma aspartate

concentrations remained within the normal range even after bolus doses of aspartame as high as 200 mg/kg. The doses of aspartame used in studies with the other subpopulations have ranged from 4 to 100 mg/kg body wt. Plasma phenylalanine concentrations observed after large-bolus and repeated doses of aspartame were comparable to the normal postprandial range in both healthy adults and PKUH and well below those in untreated PKU. Blood methanol concentrations were not detectable after aspartame doses as high as 34 mg/kg body wt when ingested as a single bolus or about 70 mg/kg body wt when administered as eight divided doses at hourly intervals. Regardless of dose, blood formate concentrations did not change from baseline levels after aspartame administration. Evaluations of urinary formate excretion after single bolus doses of aspartame as high as 200 mg/kg body wt demonstrated the body's ability to rapidly metabolize methanol and excrete formate. Thus, even when administered at levels well above 90th percentile average daily consumption (Butchko and Kotsonis, 1991, 1996), the risk of adverse effects from aspartame or its metabolic components is negligible, strongly supporting the safety of aspartame under its intended conditions of use.

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Aspartate and Excitotoxicity

Introduction

Aspartate and glutamate are some of the most common amino acids in the diet. For example, a 100-g portion of chicken provides about 2600 mg of aspartate, whereas 355 ml of beverage sweetened 100% with aspartame provides only about 70 mg of aspartate (Fig. 1). As noted under Intake of Aspartame vs the Acceptable Daily Intake, aspartame (90th percentile intake) provides only about 2 and 1% of the usual daily dietary intake of aspartate by adults and 4-year-old children, respectively. Based on metabolism studies with aspartame, it is simply not possible for a human to consume enough aspartame in products to produce a significant increase in plasma aspartate concentrations. In addition, both aspartic acid and glutamic acid are readily and rapidly metabolized and utilized by the body for energy and for incorporation into proteins.

Nonetheless, some authors (Olney, 1982; Reif-Lehrer, 1976) speculated that the aspartate constituent of aspartame, especially when consumed with foods containing monosodium glutamate (MSG), would cause an increase in the combined plasma concentrations of aspartate and glutamate, which might pose a risk of focal brain lesions. Blaylock (1993) has continued to raise this speculation.

Studies in Animals

Very high plasma concentrations of the abundant, naturally occurring dicarboxylic amino acids, aspartate and glutamate, are known to induce hypothalamic neuronal necrosis in neonatal rodents (Olney, 1969; Lemkey-Johnson and Reynolds, 1974; Okaniwa *et al.*, 1979; Finkelstein *et al.*, 1983; Daabees *et al.*, 1985). Aspartame administered in amounts ranging from 1000–2500 mg/kg body wt to neonatal mice also produced

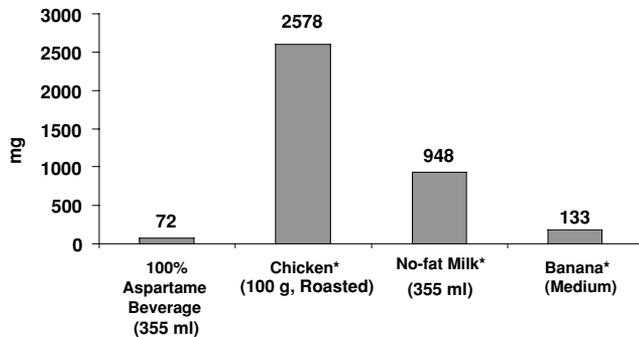


FIG. 1. Aspartic acid content of various foods and beverages compared to a 100% aspartame-sweetened beverage. Only a small amount of aspartate is provided by aspartame in products compared to that provided by common foods and beverages. *Source: U.S. Department of Agriculture, Agricultural Research Service, 2001. USDA Nutrient Database for Standard Reference, Release 14. Nutrient Data Laboratory home page, <http://www.nal.usda.gov/foodcomp>.

neuronal necrosis (Olney, 1976; Reynolds *et al.*, 1976), presumably as a result of elevated blood aspartate concentrations.

However, aspartame doses that result in plasma aspartate concentrations less than 100 $\mu\text{mol/dl}$ do not produce hypothalamic neuronal necrosis, even in the acutely sensitive neonatal mouse. For example, effects are seen only when the plasma concentration of aspartate exceeds 110 $\mu\text{mol/dl}$, the plasma concentration of glutamate exceeds 75 $\mu\text{mol/dl}$, or the combined glutamate plus aspartate concentrations exceed 128 $\mu\text{mol/dl}$ (Finkelstein *et al.*, 1983; Daabees *et al.*, 1985).

Olney and co-workers (Olney and Sharpe, 1969; Olney *et al.*, 1972) also reported this phenomenon in neonatal nonhuman primates administered large bolus doses of glutamate. This observation, however, could not be reproduced by a number of other scientists with either glutamate or aspartame at high dosages (Newman *et al.*, 1973; Wen *et al.*, 1973; Abraham *et al.*, 1975; Reynolds *et al.*, 1976, 1979, 1980). For example, aspartame (2000 mg/kg body wt) administered to neonatal nonhuman primates, with or without added MSG (1000 mg/kg body wt), did *not* produce neuronal necrosis even though plasma aspartate and glutamate concentrations were markedly elevated (Reynolds *et al.*, 1976, 1980).

Studies in Humans

As described in detail under the section Metabolism of Aspartame, Stegink and co-workers (1977, 1979) found no significant changes in plasma concentrations of aspartate after bolus doses of 34 or 50 mg/kg body wt aspartame. Significant increases in plasma aspartate concentrations were observed after aspartame doses of 100 mg/kg body wt (equivalent to the amount of aspartame in approximately 12 liters of 100% aspartame-sweetened beverage for a 60-kg individual) or higher (Stegink *et al.*, 1981a). Even with acute administration of 200 mg aspartame/kg body wt, mean peak plasma aspartate concentrations ($0.76 \pm 0.57 \mu\text{mol/dl}$) remained well below those associated with toxicity in neonatal rodents (110 $\mu\text{mol/dl}$). Evaluations of the effect of large doses (34, 50, and 100 mg/kg body wt) of aspartame (Filer *et al.*, 1983) in 1-year-old infants demonstrated that infants metabolize aspartame as well as adults. Further, daily administration of large doses of aspartame (75 mg/kg body wt/day) over 24 weeks had no significant effect on mean fasting plasma aspartate concentrations (Leon *et al.*, 1989). Thus, even extreme doses of aspartame are metabolized rapidly and do not pose a risk of adverse effects from aspartate.

The ingestion of large amounts of MSG has been claimed to produce a variety of symptoms (e.g., burning sensation, facial pressure, and chest pain) in sensitive

individuals, usually called "Chinese restaurant syndrome." Concern was expressed regarding the consumption of aspartame with MSG in foods (Olney, 1982; Reif-Lehrer, 1976), suggesting that aspartame might cause symptoms in subjects thought to be sensitive to MSG because of the structural similarity between glutamate and aspartate and the similar neurotoxicity observed at extremely large doses in animal studies. It was also suggested that the combined plasma concentrations of glutamate and aspartate (glutamate plus aspartate) may be an indicator of the risk of toxicity.

However, Stegink *et al.* (1981b) demonstrated that subjects who allegedly experienced idiosyncratic reactions to MSG do not experience similar symptoms after ingestion of either aspartame or sucrose. Stegink *et al.* (1982) also demonstrated that ingestion of aspartame (34 mg/kg body wt) plus MSG (34 mg/kg body wt) with a high protein (1 g/kg body wt) meal in healthy adults had little effect on plasma concentrations of either aspartate or glutamate beyond those from the meal alone. In another study, Stegink *et al.* (1983) demonstrated that ingestion of MSG (150 mg/kg body wt) with a high-protein meal significantly increased mean peak plasma glutamate plus aspartate concentrations above those from the meal alone; the addition of aspartame (23 mg/kg body wt) to the meal with MSG did not further increase mean peak plasma glutamate plus aspartate concentrations over the meal plus MSG. Stegink *et al.* (1987) also evaluated the effect of MSG (50 mg/kg body wt) and MSG (50 mg/kg body wt) plus aspartame (34 mg/kg body wt) on plasma amino acid concentrations when given a low-protein meal. The addition of MSG or MSG plus aspartame to the meal resulted in significant increases in plasma glutamate, aspartate, and glutamate plus aspartate concentrations over the meal alone. Although the addition of aspartame to MSG resulted in a small but significant increase in plasma aspartate concentrations over the meal plus MSG, the area under the curve (AUC) for plasma aspartate plus glutamate concentrations did not differ between the MSG compared to MSG plus aspartame conditions. The results of these studies demonstrate that it is simply impossible for a human to ever consume enough aspartame or MSG and aspartame together to raise plasma concentrations to those associated with neurotoxicity in neonatal mice. Thus, the data do not support the contention that aspartame consumed with MSG would result in rapid and dangerous rises in plasma aspartate and glutamate concentrations.

Conclusion

Thus, based on extensive metabolism data for excitatory amino acids in humans (reviewed by Meldrum, 1993), it is not possible for a human to consume enough aspartame or MSG and aspartame together in food and drink products to raise plasma concentrations of aspar-

tate or glutamate or glutamate plus aspartate to those associated with toxicity in neonatal rodents. Furthermore, given the totality of the data in primates, even if such increased plasma concentrations were possible after very large amounts of aspartame, these doses are of questionable relevance to consumption of aspartame by humans.

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Phenylalanine and Neurochemistry

Introduction

Phenylalanine is an essential amino acid required for normal growth and development and maintenance of life. However, individuals with the rare genetic disease phenylketonuria (PKU) must be placed on special diets severely restricted in phenylalanine content shortly after birth in order to avoid the markedly increased plasma phenylalanine concentrations associated with well-known sequelae of PKU, including mental retardation or various degrees of cognitive impairment. For healthy individuals and individuals heterozygous for PKU (PKUH), there is no need for dietary restriction of phenylalanine.

As aspartame is a source of phenylalanine, questions arose regarding the impact of aspartame on plasma phenylalanine concentrations (see Metabolism of Aspartame section). From the metabolism studies with aspartame, amounts of aspartame about 10 times the amount that people consume at the 90th percentile result in plasma phenylalanine concentrations within the normal postprandial range in both healthy individuals and PKUH individuals.

In addition, the typical diet provides much greater amounts of phenylalanine than provided by aspartame. For example, a serving of no-fat milk provides about six times more phenylalanine than an equivalent volume of beverage sweetened 100% with aspartame (Fig. 1). Further, aspartame provides only about 3 and 1% of daily dietary intake of phenylalanine for an adult and a 4-year-old child, respectively, at the 90th percentile of aspartame intake (see Intake of Aspartame vs the Acceptable Daily Intake section).

Nonetheless, some claims regarding aspartame have centered on possible effects of phenylalanine from as-

partame on brain function, e.g., headaches, seizures, and changes in behavior, cognition, and mood. The underlying hypothesis was that aspartame, as a source of phenylalanine (Phe) without the other large neutral amino acids (LNAAs) (i.e., tyrosine, tryptophan, valine, leucine, isoleucine, methionine, histidine) that compete for transport across the blood-brain barrier, would increase the serum ratio of phenylalanine to the other large neutral amino acids (Phe/LNAA), thereby selectively increasing brain phenylalanine concentrations. It was further hypothesized that such increased entry of phenylalanine into the brain, along with decreased entry of tyrosine and tryptophan, may result in disturbances in brain neurotransmitter concentrations (Wurtman, 1983). Such neurochemical changes had been reported in rodents (Yokogoshi *et al.*, 1984), and there were several case reports describing a potential association between seizures and aspartame consumption (Wurtman, 1985; Walton, 1986).

Upon dietary ingestion of protein and its digestion, amino acids are absorbed across the gastrointestinal mucosa into the portal circulation. In the liver, the enzyme phenylalanine hydroxylase converts phenylalanine to tyrosine. In humans, most of the dietary phenylalanine passes through the portal circulation and the liver unchanged into the systemic circulation. Once in the systemic circulation, phenylalanine is taken up across the blood-brain barrier into the central nervous system through a transport system that is relatively specific for LNAAs (Fernstrom and Wurtman, 1972). Therefore, the relative concentrations of these amino acids, together with their specific affinity constants to the carrier system, will ultimately determine how much of a given amino acid will enter and leave the brain.

In that context, more plasma phenylalanine compared to the other LNAAs will increase Phe/LNAA. Under these circumstances, phenylalanine may thereby interfere with the availability of tyrosine and tryptophan in the brain and also may act as a competitive inhibitor of the enzyme tyrosine hydroxylase (Ikeda *et al.*, 1967). As a result, this may lead to decreases in brain catecholamine and serotonin concentrations (Maher and Wurtman, 1987).

Phe/LNAA after aspartame ingestion have been evaluated in several studies (Caballero *et al.*, 1986; Møller, 1991; Stegink and Filer, 1996a,b). For example, the effect of an aspartame dose of 10 mg/kg body wt (sweetness equivalent of approximately 120 g of sucrose) on plasma phenylalanine concentrations and Phe/LNAA ratios were evaluated in subjects with widely varying capacities to metabolize phenylalanine: healthy controls, PKU heterozygotes, mild hyperphenylalaninemics, and untreated PKU homozygotes (Caballero *et al.*, 1986). Aspartame resulted in increases in plasma phenylalanine concentrations in controls

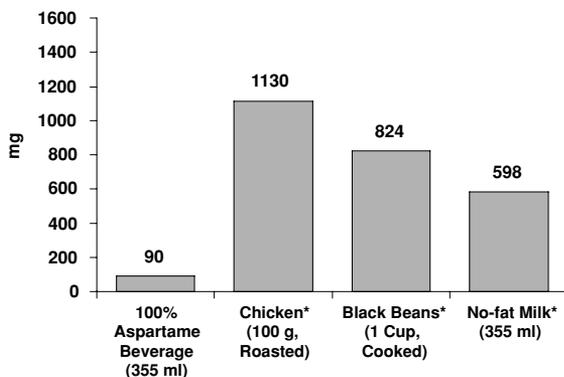


FIG. 1. Phenylalanine content of various foods and beverages compared to a 100% aspartame-sweetened beverage. Aspartame in products provides a small amount of phenylalanine compared to other dietary sources of phenylalanine. *Source: U.S. Department of Agriculture, Agricultural Research Service, 2001. USDA Nutrient Database for Standard Reference, Release 14. Nutrient Data Laboratory home page, <http://www.nal.usda.gov/fnic/foodcomp>.

(from 4.45 ± 1.29 to 5.80 ± 0.95 $\mu\text{mol/dl}$) and in PKU heterozygotes (from 6.88 ± 1.38 to 8.23 ± 1.73 $\mu\text{mol/dl}$) but did not increase the already elevated concentrations in mild hyperphenylalaninemics and untreated PKU homozygotes. Baseline Phe/LNAA ratios in these subjects were 0.100, 0.170, 0.849, and 4.212, respectively. One hour after administration of aspartame, these values were 0.137, 0.214, 0.892, and 3.847, respectively. The small increases observed in the controls and PKU heterozygotes, although statistically significant, lack biological relevance.

Fluctuations in Phe/LNAA occur as a result of dietary manipulations or normal dietary variability. For example, sugar or other carbohydrates result in changes in Phe/LNAA (Martin-Du Pan *et al.*, 1982; Stegink *et al.*, 1987; Wolf-Novak *et al.*, 1990; Burns *et al.*, 1991). In this case, the consequent insulin release, which is known to enhance the transport of amino acids across muscle membranes, decreases plasma amino acid concentrations, especially the LNAAs valine, leucine, and isoleucine. Phenylalanine from aspartame will increase the numerator, whereas insulin will result in a decrease in the denominator of Phe/LNAA. The net effect after equisweet amounts of sugar and aspartame is similar in magnitude. Thus, even if there are small changes in Phe/LNAA after aspartame, such changes are not unique to aspartame.

Considering the above data and assuming that there is no dramatic effect due to accommodation, the threshold for adverse effects, based on the Phe/LNAA ratio, can be estimated to be substantially higher than that observed in mild hyperphenylalaninemics, i.e., 0.85. Mild hyperphenylalaninemics are clinically normal and do not require phenylalanine-restricted diets. Thus, as with absolute plasma phenylalanine concentrations, the aspartame dosage required to elevate the Phe/LNAA ratio above that associated with adverse effects far exceeds that possible from consumption of products sweetened with aspartame.

It was also hypothesized that the most prolonged disturbance of the Phe/LNAA ratio would likely occur after the consumption of large doses of aspartame together with a load of carbohydrates (Yokogoshi *et al.*, 1984). In rodent studies, it was observed that large doses of aspartame may attenuate carbohydrate-induced increases in brain tryptophan and serotonin concentrations (Yokogoshi *et al.*, 1984; Fernstrom *et al.*, 1986).

However, Romano *et al.* (1989) evaluated the effect of both acute and subacute dosing of aspartame (up to 250 mg/kg body wt/day), with and without carbohydrate, on plasma and brain LNAA and brain neurotransmitter concentrations in rats. Although there were changes in plasma and brain amino acid concentrations in the acute dosing paradigm, there were no differences in brain LNAA concentrations after aspartame compared to aspartame plus carbohydrate in the subacute study. There were no effects of aspartame on brain

serotonin, 5-hydroxyindole acetic acid (5-HIAA), norepinephrine, γ -aminobutyric acid (GABA), or dopamine and its metabolites homovanillic acid (HVA) or 3,4-dihydroxyphenylacetic acid (DOPAC).

In humans, Romano *et al.* (1989) also evaluated the effect of doses of aspartame that are more relevant to human consumption (0.83 and 8.3 mg/kg body wt or the amount in up to 1 liter of beverage sweetened 100% with aspartame for a 60-kg individual), with a low or high carbohydrate meal, on plasma LNAA concentrations. There was no significant difference when aspartame was given alone or with carbohydrate. To evaluate this issue further, Wolf-Novak *et al.* (1990) compared the effect on plasma large amino acids concentrations and Phe/LNAA in humans after unsweetened beverage, beverage with 60 g carbohydrate (the amount of carbohydrate in a snack, such as a piece of cake with ice cream), beverage with aspartame (200 mg or approximately the amount in one 355 ml can of beverage), and beverage with 60 g of carbohydrate plus 200 mg of aspartame in both normal adults and PKU individuals. There were no effects of beverage with aspartame on plasma phenylalanine or other LNAA concentrations. However, the beverage with carbohydrate, with or without aspartame, resulted in decreases in plasma concentrations of valine, isoleucine, and leucine due to the insulin response after carbohydrate, resulting in an increase in Phe/LNAA. The authors concluded that the changes in Phe/LNAA were due to carbohydrate and not aspartame. Stegink *et al.* (1990) evaluated the effect of aspartame (40 mg/kg body wt) vs aspartame plus sucrose (1.2 g/kg body wt) on plasma amino acid concentrations and Phe/LNAA. Plasma phenylalanine concentrations and 4-h AUC increased after both treatments but did not differ significantly. Mean peak Phe/LNAA did not differ between treatments, and the 4-h AUC was only slightly but significantly higher after aspartame plus sucrose vs aspartame. The authors concluded that simultaneous ingestion of aspartame and sucrose had only minor effects on plasma Phe/LNAA. Thus, based on both animal and human data, there is no significant effect of aspartame at doses relevant to actual consumption on Phe/LNAA even when given with carbohydrate.

Another important consideration in the overall impact of a given phenylalanine load in studies in animals is the fact that interspecies differences exist in the extent of its hydroxylation to tyrosine. In rodents, the rate of liver conversion of phenylalanine to tyrosine is greater than the rate of conversion in humans. Therefore, in rodents, more of the dietary phenylalanine is presented to the systemic circulation as tyrosine when compared to humans, which may result in an increased uptake of tyrosine across the blood-brain barrier and thereby raise the concentration and availability of tyrosine. Wurtman and Maher (1987) postulated that because rodents metabolize phenylalanine to tyrosine faster than humans and as tyrosine may act

as an antidote to the effects of phenylalanine on brain neurochemistry, brain phenylalanine flux may be better estimated from a new ratio, $\text{Phe}_t/\text{Phe}_o/\text{Tyr}_t/\text{Tyr}_o$, which is based on relative changes in the phenylalanine (Phe) and tyrosine (Tyr) concentrations. From this, the authors estimated that there may be a 60-fold difference in this rate between rodents and humans, thus questioning whether the doses of aspartame used in animal studies of effects of aspartame on brain neurotransmitters and brain function were sufficiently large enough to account for this difference in metabolism. However, Fernstrom's (1989) evaluation of this hypothesis revealed that it is "not consistent with well-established notions of competitive LNAA transport." He concluded, "An analysis of the logic behind this postulation reveals there to be *no* basis for accepting the higher dose conversion of 60 between rat and man."

Further, in a study done to evaluate this hypothesis, it was shown that rodents require a dose of aspartame only two to six times higher than humans to produce similar increases in plasma Phe/LNAA (Hjelle *et al.*, 1992). Thus, even with this difference in metabolism, the doses of aspartame (up to 3000 mg/kg body wt) used in the animal studies of effects of aspartame on brain neurotransmitters, seizures, behavior, etc., are very large compared to doses evaluated in humans (up to 200 mg/kg body wt) as a bolus and 1000 times 90th percentile average daily consumption of aspartame (3 mg/kg body wt) in the United States (Butchko and Kotsonis, 1991).

In addition to the studies in animals and hypotheses, Koeppe *et al.* (1991) evaluated amino acid uptake into the brain in humans using positron emission scanning. These authors demonstrated an 11.5% decrease in amino acid transport rate constant into the brain after a large (34 mg/kg body wt) oral dose of aspartame (about 10 times 90th percentile daily consumption of aspartame). Based on this, the authors concluded that there would be little measurable transport change under more relevant conditions of aspartame use.

Studies of Aspartame and Adrenergic, Serotonergic, and Dopaminergic Neurotransmitter Systems

A number of animal studies have been done to determine whether plasma phenylalanine concentration increases secondary to aspartame loading may result in changes in concentration or turnover of the neurotransmitters, norepinephrine, dopamine, or serotonin, and their metabolites in whole brain or specific regions of the brain (Table 1) (Fernstrom *et al.*, 1983; Yokogoshi *et al.*, 1984; Yokogoshi and Wurtman, 1986; Coulombe and Sharma, 1986; Sharma and Coulombe, 1987; Perego *et al.*, 1988; Garattini *et al.*, 1988; Reilly *et al.*, 1989a, 1990; Romano *et al.*, 1989, 1990; Dailey *et al.*, 1989, 1991; Freeman *et al.*, 1990; Diomedea *et al.*, 1991; Helali *et al.*, 1996; Goerss *et al.*, 2000). A compre-

hensive review of the studies on aspartame and neurochemistry has been published by Lajtha *et al.* (1994).

Most of these studies were acute dosing paradigms (Fernstrom *et al.*, 1983; Yokogoshi *et al.*, 1984; Yokogoshi and Wurtman, 1986; Coulombe and Sharma, 1986; Perego *et al.*, 1988; Garattini *et al.*, 1988; Romano *et al.*, 1989, 1990; Dailey *et al.*, 1989, 1991; Freeman *et al.*, 1990; Diomedea *et al.*, 1991; Helali *et al.*, 1996; Goerss *et al.*, 2000), and a few were subacute (Romano *et al.*, 1989; Helali *et al.*, 1996) or subchronic (Sharma and Coulombe, 1987; Reilly *et al.*, 1989a; Dailey *et al.*, 1991). In the acute dosing studies, the doses varied from 13 to 2500 mg/kg body wt. Most studies were oral/gavage-dosing paradigms, whereas some (e.g., Goerss *et al.*, 2000) used intraperitoneal (ip) dosing. In the subacute dosing studies (9–14 days), doses ranged up to 250 mg/kg body wt/day. In the subchronic dosing studies (up to 30 days), doses of aspartame ranged up to 863 mg/kg body wt/day.

In most of these studies, concentrations of monoamine neurotransmitters and their metabolites were measured in structures such as the cortex, hippocampus, striatum, midbrain, thalamus, and nucleus accumbens. Whole brain concentrations of the neurotransmitters and metabolites were evaluated in other studies. While isolated effects of aspartame on brain neurotransmitter or neurotransmitter metabolite concentrations were noted in a few studies (Yokogoshi and Wurtman, 1986; Coulombe and Sharma, 1986; Sharma and Coulombe, 1987; Dailey *et al.*, 1989, 1991; Freeman *et al.*, 1990; Helali *et al.*, 1996; Goerss *et al.*, 2000), it was clear that these effects were neither consistent nor reproducible. For the most part, despite large increases in brain phenylalanine concentrations and increases in Phe/LNAA, about half of the few reported changes in brain concentrations of neurotransmitters or their metabolites were increases rather than the hypothesized decreases.

In addition, in 30-day subchronic studies (Reilly *et al.*, 1989b, 1990; Reilly and Lajtha, 1995a), aspartame (up to 500 mg/kg body wt/day) had no effect on adrenergic, serotonergic, or dopaminergic receptor kinetics in young adult rats or in rats exposed to aspartame during gestation and lactation. In an acute *in vivo* voltammetry study in freely moving rats (Perego *et al.*, 1988; Garattini *et al.*, 1988), recordings for DOPAC in the striatum and the nucleus accumbens and for 5-HIAA in these same brain areas as well as the hippocampus were no different in rats given 1000 mg/kg body wt of aspartame compared to control. Further, a study using transstriatal dialysis demonstrated no significant effect of phenylalanine (100 and 450 mg/kg body wt) on release of dopamine or levels of its metabolites in either rat or baboon striatum (Arvin *et al.*, 1992). Such studies are considered more reliable in evaluating changes in neurotransmission than steady state neurotransmitter concentrations. All these studies failed to show a

TABLE 1
Brain Neurotransmitter and Neurotransmitter Metabolite Concentrations
in Animals after Aspartame Dosing

	No changes	Increased	Decreased
Norepinephrine	Fernstrom <i>et al.</i> (1983) Yokogoshi and Wurtman (1986) Sharma and Coulombe (1987) Perego <i>et al.</i> (1988) Romano <i>et al.</i> (1989) Reilly <i>et al.</i> (1989a) Dailey <i>et al.</i> (1989) Freeman <i>et al.</i> (1990) Reilly <i>et al.</i> (1990) Diomede <i>et al.</i> (1991) Helali <i>et al.</i> (1996)	Yokogoshi and Wurtman (1986) Coulombe and Sharma (1986) Dailey <i>et al.</i> (1991)	Yokogoshi and Wurtman (1986) Helali <i>et al.</i> (1996)
Dopamine	Fernstrom <i>et al.</i> (1983) Yokogoshi and Wurtman (1986) Sharma and Coulombe (1987) Garattini <i>et al.</i> (1988) Perego <i>et al.</i> (1988) Romano <i>et al.</i> (1989) Reilly <i>et al.</i> (1989a) Dailey <i>et al.</i> (1989) Romano <i>et al.</i> (1990) Freeman <i>et al.</i> (1990) Reilly <i>et al.</i> (1990) Diomede <i>et al.</i> (1991) Goerss <i>et al.</i> (2000)	Yokogoshi and Wurtman (1986) Coulombe and Sharma (1986) Dailey <i>et al.</i> (1991)	
Serotonin	Fernstrom <i>et al.</i> (1983) Yokogoshi <i>et al.</i> (1984) Coulombe and Sharma (1986) Perego <i>et al.</i> (1988) Garattini <i>et al.</i> (1988) Romano <i>et al.</i> (1989) Reilly <i>et al.</i> (1989a) Reilly <i>et al.</i> (1990) Freeman <i>et al.</i> (1990) Diomede <i>et al.</i> (1991)	Sharma and Coulombe (1987) Goerss <i>et al.</i> (2000)	Sharma and Coulombe (1987) Dailey <i>et al.</i> (1989) Dailey <i>et al.</i> (1991)
5HIAA ^a	Yokogoshi <i>et al.</i> (1984) Coulombe and Sharma (1986) Romano <i>et al.</i> (1989) Perego <i>et al.</i> (1988) Freeman <i>et al.</i> (1990) Diomede <i>et al.</i> (1991) Fernstrom <i>et al.</i> (1983) Garattini <i>et al.</i> (1988) Reilly <i>et al.</i> (1989a) Reilly <i>et al.</i> (1990) Goerss <i>et al.</i> (2000)	Goerss <i>et al.</i> (2000)	Dailey <i>et al.</i> (1989) Sharma and Coulombe (1987) Goerss <i>et al.</i> (2000)
HVA ^a	Fernstrom <i>et al.</i> (1983) Sharma and Coulombe (1987) Garattini <i>et al.</i> (1988) Perego <i>et al.</i> (1988) Romano <i>et al.</i> (1989) Reilly <i>et al.</i> (1989a) Freeman <i>et al.</i> (1990) Romano <i>et al.</i> (1990) Reilly <i>et al.</i> (1990) Diomede <i>et al.</i> (1991) Goerss <i>et al.</i> (2000)	Coulombe and Sharma (1986)	
Dopa	Fernstrom <i>et al.</i> (1983)		

TABLE 1—Continued

	No changes	Increased	Decreased
DOPAC ^a	Fernstrom <i>et al.</i> (1983) Perego <i>et al.</i> (1988) Garattini <i>et al.</i> (1988) Romano <i>et al.</i> (1989) Reilly <i>et al.</i> (1989a) Romano <i>et al.</i> (1990) Reilly <i>et al.</i> (1990) Diomedea <i>et al.</i> (1991) Goerss <i>et al.</i> (2000)	Yokogoshi and Wurtman (1986) Coulombe and Sharma (1986) Freeman <i>et al.</i> (1990)	Yokogoshi and Wurtman (1986) Sharma and Coulombe (1987)
VMA ^a	Sharma and Coulombe (1987)	Coulombe and Sharma (1986)	
MHPG ^a	Yokogoshi and Wurtman (1986) Garattini <i>et al.</i> (1988) Romano <i>et al.</i> (1989)	Yokogoshi and Wurtman (1986)	
GABA ^a	Romano <i>et al.</i> (1989) Helali <i>et al.</i> (1996)		Helali <i>et al.</i> (1996)
Epinephrine	Yokogoshi and Wurtman (1986) Helali <i>et al.</i> (1996)	Yokogoshi and Wurtman (1986)	Yokogoshi and Wurtman (1986) Helali <i>et al.</i> (1996)
Aspartate	Reilly and Lajtha (1995a) Reilly and Lajtha (1995b) Helali <i>et al.</i> (1996)	Helali <i>et al.</i> (1996)	Reilly and Lajtha (1995a) Reilly and Lajtha (1995b)
Glutamate	Helali <i>et al.</i> (1996)		Reilly and Lajtha (1995a) Reilly and Lajtha (1995b)

^a 5HIAA, 5-hydroxyindole acetic acid; HVA, homovanillic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; VMA, 3-methoxy-4-hydroxymandelic acid; MHPG, 3-methoxy-4-hydroxyphenylethylene glycol; GABA, γ -aminobutyric acid.

treatment effect of large doses of aspartame or phenylalanine.

The potential effect of aspartame on neurotransmitter systems in the developing brain, which was thought to be more "sensitive," was also studied. Rats were exposed to aspartame administered to dams throughout gestation and lactation. No effects were observed on adrenergic, serotonergic, or dopaminergic neurotransmitter concentrations or neurotransmitter receptor kinetics in the dams or the weanlings (Reilly *et al.*, 1990; Reilly and Lajtha, 1995a).

In an *in vitro* study, Fountain *et al.* (1988) noted that aspartame, aspartic acid, phenylalanine, and phenylalanine methyl ester potentiated the response of hippocampal CA 1 pyramidal cells; however, there was no effect on inhibitory systems or the processes underlying synaptic plasticity. (Suppression of inhibitory processes is commonly associated with convulsants.) The authors concluded that aspartame and its moieties "are not likely to produce significant neurotoxic effects at physiologically realistic acute doses."

Further, regarding the conjecture that phenylalanine from aspartame may inhibit tyrosine hydroxylase activity and thereby impact brain monoaminergic neurotransmitters, Fernstrom *et al.* (1991) evaluated the effects of large doses of aspartame up to 1500 mg/kg body wt on the rate of tyrosine hydroxylation in rat retina. In this study, rats were pretreated with ρ -chlorophenylalanine to inhibit hepatic metabolism of

phenylalanine to tyrosine, and thereby more closely mimic the human condition. Although the concentration of retinal phenylalanine increased about sixfold, there were no significant changes in tyrosine concentrations and no changes in the rate of tyrosine hydroxylation or dopamine concentrations in the retina. Thus, enormous doses of aspartame did not inhibit or stimulate the *in vivo* rate of tyrosine hydroxylation in the retina.

Studies of the Aspartate and Glutamate Neurotransmitter Systems

Helali *et al.* (1996) observed increased brain aspartate concentrations in mice after acute aspartame dosing with 1000 mg/kg body wt but no changes in brain glutamate or GABA concentrations. However, with subacute dosing (100 mg/kg body wt/day for 14 days), these authors found no changes in brain aspartate or glutamate concentrations but a significant reduction in GABA. In another study, Romano *et al.* (1989) found no effect of aspartame on rat cortical GABA concentrations, with or without a carbohydrate-enriched diet, over 14 days. From an *in vitro* study, Pan-Hou *et al.* (1990) suggested that aspartame, and especially its aspartate component, inhibited glutamate binding to *N*-methyl-D-aspartate (NMDA) receptors in a dose-dependent manner, altering the affinities for the receptors without altering V_{max} . From another *in vitro* study,

Sonnewald *et al.* (1995) reported a dose-dependent increase in calcium influx and leakage of lactate dehydrogenase from murine brain cell cultures, which he felt confirmed the hypothesis of Pan-Hou regarding effects on the NMDA receptor. However, the relevance of such *in vitro* work is questionable since brain cells are not exposed to intact aspartame after consumption given its rapid metabolism to its three components before absorption. In contrast, Reilly and Lajtha (1995a,b) evaluated the effect of aspartame 500 mg/day for 30 days on NMDA and total glutamatergic receptor binding kinetics in adult dams and weanlings, which had been exposed to aspartame throughout gestation and lactation. Although there were small but reversible decreases in brain concentrations of glutamic acid (cortex and hippocampus) and aspartic acid (cortex only) in weanlings, there were no effects on the kinetics of binding to the NMDA receptor and total glutamate binding in the cerebral cortex and hippocampus after perinatal exposure to high doses of aspartame.

Conclusion

Numerous studies have been done to evaluate the hypothesis that aspartame, as a source of phenylalanine without the other large neutral amino acids that compete with it for entry into the brain, may result in changes in brain neurotransmitters, which may thereby alter brain function. It was clear from these studies that there was no consistent pattern of such effects nor was there evidence of reproducibility. For the most part, despite large increases in brain phenylalanine concentrations, about half of the few reported changes in brain catecholamine concentrations were increases rather than the hypothesized decreases. Further, there was no effect on receptor kinetics after very large doses of aspartame in either adult animals or weanlings exposed to aspartame throughout gestation and lactation or on release of neurotransmitters. There also were no consistent effects on brain glutamate, aspartate, or GABA concentrations or on binding to NMDA receptors. Thus, it is concluded that consumption of aspartame does not adversely affect neurochemical function of the brain.

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Safety of Methanol from Aspartame and the Diet

Introduction

Aspartame metabolism yields approximately 10% methanol by weight, and the safety of methanol from aspartame has been evaluated in numerous studies. The extensive database of safety studies with aspartame includes studies in both laboratory animals and humans, and 10% of the extremely large doses administered can be considered to be methanol. The battery of preclinical toxicology studies consists of numerous toxicity studies, including four 2-year or lifetime studies in rodents and including one in rats with *in utero* exposure followed by 104 weeks of testing, as well as reproduction, teratology, and mutagenicity studies (Kotsonis and Hjelle, 1996). The no-observed-effect dose of aspartame was at least 4000 mg/kg body wt/day as established by the Joint FAO/WHO Expert Committee on Food Additives (1980), the Scientific Committee for Food (1985), and the Health Protection Branch of Health and Welfare Canada (1979). This dose of aspartame provides 400 mg/kg body wt/day of methanol and would be the equivalent of a 60-kg human consuming 24,000 mg of methanol every day over a lifetime.

When evaluating potential toxicity of any substance, the actual dose of the substance is of major importance. In the case of aspartame, a number of intake studies have been done over the past 15 years. The most extensive evaluation of aspartame intake was in the United States from 1984 to 1992 where intake data were evaluated over 14-day periods from about 5000 people per year. In the United States, aspartame intake by the general population at the 90th percentile is about 3.0 mg/kg body wt/day (Butchko and Kotsonis, 1991, 1994, 1996; Butchko *et al.*, 1994; Butchko and Stargel, 2001), well below the acceptable daily intake (ADI) in the United States (50 mg/kg body wt/day) and in Europe (40 mg/kg body wt/day). Taking into account differences in methodologies, the results of intake studies from other countries, including a number of European countries, have been remarkably consistent with the U.S. data (Hinson and Nicol, 1992; MAFF, 1990, 1995; Bar and Biermann, 1992; Chambolle *et al.*, 1994; Garnier-Sagne *et al.*, 2001; Virtanen *et al.*, 1988; Leclercq *et al.*, 1999; Heybach and Ross, 1989; Bergsten, 1993; Hulshof and Bouman, 1995; National Food Authority Australia, 1995; Toledo and Ioshi, 1995). Thus, methanol exposure from aspartame at the 90th percentile of aspartame intake in the United States is only 0.3 mg/kg body wt/day or about 1/13–1/16 of the amount of methanol provided by aspartame at the ADIs in the US and Europe.

Pathogenesis of Methanol Toxicity: The Importance of Formate Accumulation

The classic pattern of methanol toxicity includes an initial central nervous system (CNS) depression weaker

than that produced by ethanol, followed by a latent period of 10–30 h. After this asymptomatic period, a syndrome develops that is characterized specifically by visual impairment (ranging from blurring to total loss of vision) and severe metabolic acidosis. In severe cases, CNS depression and coma can ensue, with death possible from respiratory depression.

There is a distinct lack of correlation between blood methanol levels and the degree of toxicity, suggesting that methanol itself is not a toxic agent. The existence of a latent period between the exposure to methanol and the onset of symptoms further suggests that a toxic metabolite is accumulating during the latent period (Tephly, 1991). Initial experimental studies in animals failed to identify the toxic metabolite(s), because they were conducted in nonprimate species that do not develop the syndrome of methanol poisoning that is seen in humans. However, monkeys have been shown to be susceptible to methanol in the same way as humans (Martin-Amat *et al.*, 1977). Studies using fomepizole (4-methylpyrazole) as an inhibitor of methanol metabolism in the monkey have confirmed that methanol is toxic because of its conversion to a toxic metabolite (McMartin *et al.*, 1975).

Methanol is initially metabolized via alcohol dehydrogenase (in humans and monkeys) to formaldehyde, a highly reactive chemical. Formaldehyde is then rapidly degraded to formate by several enzyme systems, including the specific formaldehyde dehydrogenase complex ubiquitously distributed among various tissues (Uotila and Koivusalo, 1974). Because of its extensive metabolism and high reactivity, formaldehyde is eliminated from the blood very rapidly in all studied species, with half-lives in the range of 1–2 min (McMartin *et al.*, 1979). In fact, no formaldehyde has been measured in blood or tissues of methanol-poisoned humans (Alha *et al.*, 1958) or monkeys. In monkeys, parallel studies with infusions of small doses of formaldehyde showed that the measurement techniques were sufficiently sensitive to detect formaldehyde in blood and tissues (McMartin *et al.*, 1979). Thus, methanol-induced formaldehyde accumulation in body fluids is not likely.

Formaldehyde is completely and quantitatively converted to formate. In humans and in monkeys, the accumulation of formate has been shown to be responsible for the production of metabolic acidosis in methanol poisoning (McMartin *et al.*, 1975; Sejersted *et al.*, 1983). In patients who have been intoxicated with methanol and in experimental studies in monkeys, the increase in the anion gap in the blood can be completely explained by the accumulation of formate. In species that are not sensitive to methanol such as the rat, formate accumulation and metabolic acidosis do not occur (McMartin *et al.*, 1975). Hence, the species difference in methanol sensitivity is related to the capacity to accumulate

formate. The rate of formate production appears to vary little among species; rather the accumulation of formate appears to result from differences in the rate of formate oxidation to CO₂. The rate of formate metabolism is at least 50% slower in the monkey than in the rat (McMartin *et al.*, 1977). If the rate of formate metabolism is reduced in the rat by 50%, then formate accumulation and acidosis are produced after methanol is administered (Makar and Tephly, 1976). Also, if the rate of formate metabolism is increased in the monkey, then formate accumulation and acidosis after methanol are markedly reduced (Noker *et al.*, 1980). Among various species, the primary route of metabolism of formate appears to involve the folate biochemical pathway. Formate enters the pathway by combining with tetrahydrofolate (THF) through the enzyme formyl-THF synthetase to form 10-formyl-THF, which can then be oxidized to CO₂ with regeneration of THF through the enzyme formyl-THF dehydrogenase. The rate of formate metabolism correlates well with the hepatic concentrations of THF (Black *et al.*, 1985).

The visual toxicity of methanol is an important characteristic of the overall syndrome. The mechanism for the ocular effects of methanol is not completely understood, although formate is considered to be the toxic agent. Studies in monkeys have shown that formate can produce an ocular toxicity similar to that produced after ingestion of large amounts of methanol. In these studies, formate buffers were infused to produce steady-state concentrations of formate ≥ 10 mmol/liter without any acidosis (Martin-Amat *et al.*, 1978). More recent studies in a rodent model, in which formate oxidation is selectively inhibited with nitrous oxide, have shown that formate accumulates to toxic concentrations after methanol treatment, concurrently with the development of metabolic acidosis and visual toxicity (Murray *et al.*, 1991). Studies in this model have linked formate accumulation with abnormalities in indicators of retinal function such as the electroretinogram (Eells *et al.*, 1996). A hypothesis has been formulated to explain how formate and methanol can produce ocular toxicity (Martin-Amat *et al.*, 1977). Formate is an inhibitor of cytochrome oxidase, which could inhibit ATP formation in the optic nerve and retina, leading eventually to loss of visual function. This inhibition occurs in the 5–30 mM range (Nicholls, 1976), which is similar to the formate concentrations associated with retinal toxicity in humans and primates. Formate also appears to increase retinal vulnerability to oxidative injury by causing a depletion of glutathione, which is the major endogenous molecule protecting against oxidative stress in the retina (Seme *et al.*, 2001).

Human Studies Evaluating the Safety of Methanol from Aspartame

In humans, Stegink and co-workers (1981, 1983) evaluated blood methanol and formate concentrations af-

TABLE 1
Blood Formate Concentrations (Mean \pm SD) after a Bolus Dose of 200 mg/kg Body Wt Aspartame in Healthy Adults^a

Time (h)	Blood formate (mg/dl)
Prestudy	1.19 \pm 0.61
0	2.28 \pm 1.83
0.25	1.12 \pm 0.54
0.50	1.69 \pm 1.11
0.75	1.63 \pm 1.13
1.0	1.56 \pm 0.33
1.5	1.60 \pm 0.53
2.0	1.43 \pm 0.77
3.0	2.15 \pm 1.21
4.0	1.12 \pm 0.42
5.0	1.45 \pm 0.73
6.0	1.23 \pm 0.54
7.0	0.84 \pm 0.68
8.0	1.29 \pm 0.52
24.0	1.10 \pm 1.25

^a Adapted from Stegink *et al.* (1981).

ter large bolus doses of aspartame, varying from 34, 100, 150, to 200 mg/kg body wt, in adults and 1-year-old infants. The highest dose is equal to the amount of aspartame in about 25 liters of 100% aspartame-sweetened beverage consumed at once by an adult. Despite dose-related increases in blood methanol concentrations, there was no detectable increase in blood formate (Table 1), the metabolite of methanol responsible for toxicity in humans. There was, however, at the highest dose, a small increase in urinary formate excretion (Table 2), confirming that the body is well able to handle even enormous doses of aspartame safely.

In studies with repeated doses of aspartame (600 mg aspartame every hour for 8 h) in both healthy adults and PKU heterozygotes, blood methanol concentrations were below the limit of detection (Stegink *et al.*, 1989, 1990). There was no effect on blood formate concentrations and no effect on urinary formate excretion after aspartame compared to placebo.

No differences in blood methanol and formate concentrations or urinary formate excretion were observed

TABLE 2
Urinary Formate Excretion (Mean \pm SD) after a Bolus Dose of 200 mg/kg Body Wt Aspartame in Healthy Adults^a

Urine collection interval (h)	Formate excretion (μ g/mg creatinine)
-8-0	34 \pm 22
0-4	101 \pm 30*
4-8	81 \pm 22*
8-24	38 \pm 12

^a Adapted from Stegink *et al.* (1981).

* Significantly different from baseline ($P < 0.01$).

in individuals with liver disease after a bolus dose of aspartame (15 mg/kg body wt) compared to placebo (Hertelendy *et al.*, 1993). In children with attention deficit disorder given aspartame (34 mg/kg body wt) or placebo for 2 weeks on each treatment (Shaywitz *et al.*, 1994), there was no detectable blood methanol and no differences in plasma formate or urinary formate concentrations after aspartame vs placebo.

In a study of long-term exposure to large doses of aspartame (Leon *et al.*, 1989), healthy adult subjects were given 75 mg/kg body wt of aspartame or placebo daily for 6 months (i.e., the amount of aspartame in about 10 liters of beverage sweetened with 100% aspartame for an adult). This very large dose of aspartame provides 7.5 mg/kg body wt/day methanol. During the study with measurements at 6-week intervals, most blood methanol concentrations were below the limit of detection in both the aspartame and the placebo groups. The number of subjects with detectable blood methanol concentrations was similar in both groups. Thus, there was no accumulation of methanol from long-term ingestion of these very large doses of aspartame. In addition, blood formate concentrations were not significantly increased after aspartame. Further, evaluation of 24-h urine collections revealed no increase in urinary formate excretion after aspartame compared to placebo or in urinary formate to creatinine ratio, indicating no significant increase in formate formation during high-dose, long-term aspartame intake.

Dietary Exposure to Methanol

The amount of methanol released from dietary exposure to aspartame is less than normal dietary exposure to methanol from fruits, vegetables, and juices (Butchko and Kotsonis, 1989, 1991). For example, a serving of tomato juice provides about six times more methanol than an equivalent volume of beverage sweetened 100% with aspartame (Fig. 1). Safe dietary exposure to methanol from fruits and vegetables and their juices and from alcoholic beverages has been the subject

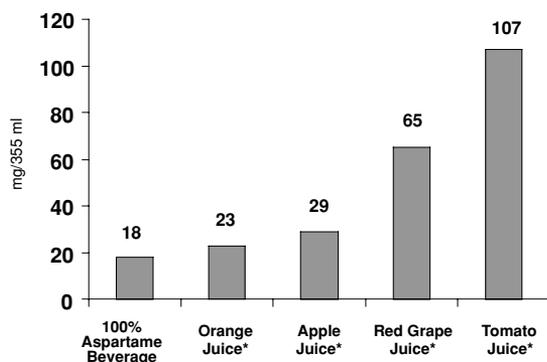


FIG. 1. Methanol derived from various fruit juices compared to that derived from aspartame in a 100% aspartame-sweetened beverage. *Source: Wucherpennig *et al.* (1983).

of investigation for a number of years with a range of methanol concentrations reported from different studies. In addition, other sources of dietary methanol in a healthy diet included filbert nuts, legumes, and vegetables that are not typically used for juices, such as potato, onion, Brussels sprouts, celery, and parsnip (WHO, 1997).

As noted by several authors, the highly variable methanol content of juices depends not only on the type of fruit but also on the ripeness of the fruit as well as the type of processing and storage time (Bindler *et al.*, 1988; Kirchner and Miller, 1957; Kirchner *et al.*, 1953). In addition, Wucherpennig *et al.* (1983) point out that methanol content of juices should be addressed from the standpoint of "total" methanol content, meaning "existing" (i.e., "free") methanol in the juice plus the "potential" (i.e., "releasable") methanol that is made available as pectin is broken down by enzymes or during storage or digestion. For example, Wucherpennig and co-workers reported for tomato juice, the "existing" methanol content of 159 mg/liter in addition to "potential" methanol content of 142 mg/liter, resulting in a "total" methanol content of 301 mg/liter. In this example, the total methanol exposure is about two times the nominal concentration of "free" methanol in tomato juice, which suggests that the methanol concentrations reported in juices in other studies may be underestimates of dietary methanol exposure.

Consistent with the approach of Wucherpennig and co-workers, a report from a panel of experts published by the World Health Organization (WHO, 1997) discussed that dietary methanol can arise from fresh fruits and vegetables as free methanol, methyl esters of fatty acids, or methoxy groups on polysaccharides such as pectin. Sommer (1962) and Grüner *et al.* (1994) have reported that pectins are metabolized in the human GI tract with consequent release and absorption of methanol. Therefore, it is likely that a healthy diet actually safely provides greater amounts of methanol than would appear to be the case from the reported methanol contents of juices.

Finally, Taucher *et al.* (1995) calculated the lower limit of the actual rate of methanol production in humans after eating fruit by evaluating methanol in human breath after fruit consumption. These authors estimated "methanol production in the human body of up to ~0.1 g/hr, showing that because of fruit consumption, the body can produce ~1 g of methanol over the time period of 1 day in addition to the background natural (physiological) base methanol production (~0.015 g/hr)." Based on these findings, significant amounts of dietary methanol from fruits are commonly and safely handled by humans.

FDA Evaluation of Methanol Safety

FDA (1994, 1996) has specifically evaluated the safety of dietary methanol intake and concluded,

“...the tolerable (safe) level of exposure to methanol is 7.1 to 8.4 milligrams per kilogram body weight per day (mg/kg body weight/day), or approximately 426 to 504 mg/person/day for a 60 kg adult.” Further, FDA also considered metabolic capacity when evaluating safe levels of methanol intake in humans. FDA (1988) stated, “An adult human can metabolize up to 1500 milligrams of methanol per hour with no adverse symptoms or effects.” Thus, the capacity for methanol metabolism in humans is far greater than estimated dietary intake. In the case of methanol exposure from aspartame, 0.3 mg/kg body wt/day at the 90th percentile, the safe level as established by FDA is about 25 times that from aspartame.

Evaluation of Recent Issues Regarding Methanol Safety from Aspartame

Trocho *et al.* (1998) concluded from a study in rats that aspartame may be hazardous because formaldehyde adducts from aspartame may accumulate in tissue proteins and nucleic acids. However, according to Tephly (1999), the dose of aspartame used in the study (20 mg/kg body wt = 2 mg of methanol/kg body wt) would not yield blood methanol concentrations outside control values. Further, the administration of aspartame at 200 mg/kg body wt (equal to that in a single bolus of about 25 liters of beverage sweetened 100% with aspartame) to adult humans results in no detectable increase in blood formate concentrations (Stegink *et al.*, 1981). Administration of [¹⁴C]methanol itself at 3000 mg/kg body wt to monkeys produces no detectable [¹⁴C]formaldehyde in body fluids and tissues (McMartin *et al.*, 1979), while there is ample accumulation of formate. An alternative explanation for tissue incorporation of label from [¹⁴C]aspartame as described by Trocho *et al.* (1998) would be incorporation into amino acids and nucleotides via one-carbon moieties from the folate-dependent metabolism of formate. The lack of formaldehyde accumulation at very high doses of methanol question considerably the conclusion that formaldehyde adducts are forming from low doses of methanol (derived from high doses aspartame). Thus, Tephly (1999) concluded, “the normal flux of one-carbon moieties whether derived from pectin, aspartame, or fruit juices is a physiologic phenomenon and not a toxic event.”

Conclusion

The safety of methanol and methanol derived from aspartame has been extensively evaluated. Based on metabolism studies, it is not possible for a human to ever consume enough aspartame in products to raise blood formate concentrations. Because formate is the toxic metabolite of methanol, the lack of formate accumulation after very large doses of aspartame indicates that the conversion of aspartame to methanol is not sufficient to induce any toxicity from methanol or its metabolites.

The enormous doses of aspartame used in the metabolism studies in humans are about 10–65 times the 90th percentile average daily intake of aspartame. Further, the amount of methanol derived from aspartame is less than the amounts derived from fruits and vegetables and their juices and alcoholic beverages in the normal diet. According to the FDA (1988), “An adult human can metabolize up to 1500 milligrams of methanol per hour with no adverse symptoms or effects.” Thus, the capacity for methanol metabolism in humans is far greater than estimated dietary intake from all sources.

FDA evaluated the safe levels of methanol intake and concluded, “...the tolerable (safe) level of exposure to methanol is 7.1 to 8.4 milligrams per kilogram body weight per day (mg/kg body weight/day), or approximately 426 to 504 mg/person/day for a 60 kg adult” (FDA, 1994, 1996). This amount of methanol is about 25 times greater than the amount of methanol (0.3 mg/kg body wt) provided by aspartame to the diet at the 90th percentile intake.

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Postmarketing Surveillance: Evaluation of Anecdotal Reports of Health Effects

Introduction

In the 1940s and 1950s, when many new drugs were being developed and marketed, it became apparent that the full spectrum of adverse reactions was not always apparent until many patients had been exposed to the drug over a period of time (Faich, 1986). Thus, it was concluded that a postmarketing surveillance system to document and evaluate spontaneous reports of any adverse reactions associated with marketed pharmaceuticals would be useful to expand the safety profile documented from the extensive preapproval studies.

Shortly after aspartame's marketing in the United States, a number of anecdotal reports of health effects, which some consumers related to their consumption of aspartame-containing products, were documented. Not unexpectedly, the numbers and types of these reports were influenced by negative media stories (CDC, 1984; Butchko *et al.*, 1996; Butchko and Stargel, 2001). The NutraSweet Company developed a postmarketing surveillance system for aspartame, based on the principles used for postmarketing surveillance of pharmaceuticals, to document and evaluate these anecdotal reports (Butchko and Kotsonis, 1989, 1994; Butchko *et al.*, 1994, 1996, 2001; Butchko and Stargel, 2001, 2002). Further, data from this system were shared with the U.S. FDA and the Centers for Disease Control (CDC), as discussed below.

Following the approval of aspartame in carbonated beverages in 1983, there was an increase in the reporting of adverse health events allegedly associated with the consumption of aspartame-containing products. This led the FDA to request the CDC to evaluate these reports (CDC, 1984; Bradstock *et al.*, 1986). In addition, the FDA's Center for Food Safety and Applied Nutrition (CFSAN) started its own process, the Adverse Reaction Monitoring System (ARMS), in 1985 to monitor accounts of health problems anecdotally associated with consumption of foods, food and color additives, and vitamin/mineral supplements (Tollefson, 1988; Tollefson *et al.*, 1988).

Evaluation by the Centers for Disease Control

CDC analyzed over 500 reports with about half undergoing detailed follow-up and evaluation. Most of the reports came from white females aged 21–60 years and were randomly distributed throughout the United States, with one exception. As aspartame had been subjected to substantial negative media coverage in Arizona, there were proportionately more reports from that state. While a variety of different symptoms were reported, two-thirds fell into the neurologic/behavioral category, consisting mostly of headache, mood alter-

ations, insomnia, and dizziness. About a quarter of the reports were gastrointestinal in nature and included abdominal pain, nausea, diarrhea, and vomiting (CDC, 1984; Bradstock *et al.*, 1986).

The CDC concluded, "Despite great variety overall, the majority of frequently reported symptoms were mild and are symptoms that are common in the general populace" (CDC, 1984). There were no specific clinical syndromes that suggested a causal relationship with aspartame. The CDC concluded that focused clinical studies would be the way to address the issues raised by the anecdotal reports.

Evaluation by Food and Drug Administration ARMS

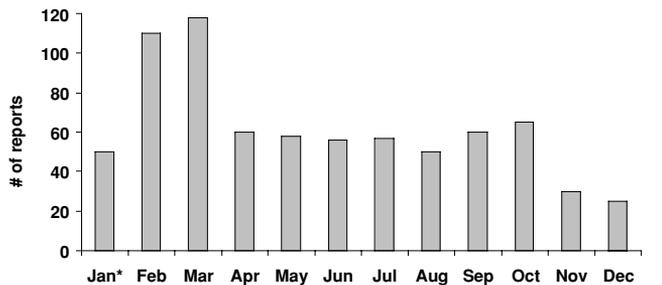
Unlike the case of pharmaceuticals, where most information is received from physicians, information regarding food additives is largely reported by consumers. In the case of aspartame, about 70% of the reports in ARMS were provided by The NutraSweet Company. Reports to ARMS are categorized based on the severity of symptoms and on the consistency and frequency with which they occur. The FDA concluded that there is no "reasonable evidence of possible public health harm" and "no consistent or unique patterns of symptoms reported with respect to aspartame that can be causally linked to its use" (Tollefson *et al.*, 1988).

In a 1995 FDA report on aspartame, a total of 7232 consumer reports had been received since marketing with only about 11% classified as serious. Headache was the most common symptom reported, followed by dizziness, mood changes, and nausea/vomiting (Table 1). The report noted that the numbers of reports from consumers regarding aspartame had declined since the

TABLE 1
Anecdotally Reported Symptoms^a

Reported symptom	Percentage of complaints
Headache	19.0
Dizziness/poor equilibrium	7.5
Change in mood	6.7
Vomiting or nausea	6.6
Abdominal pain and cramps	4.7
Change in vision	3.7
Diarrhea	3.4
Seizures and convulsions	3.0
Memory loss	2.6
Fatigue, weakness	2.5
Other neurological	2.4
Rash	2.3
Sleep problems	2.1
Hives	2.0
Change in heart rate	1.9

^a Source: FDA (1995).



* Negative series on aspartame aired on CBS TV on January 16-18, 1984

FIG. 1. Negative media reports regarding aspartame had a marked impact on the number of anecdotal reports of health effects allegedly associated with aspartame received by The NutraSweet Company in early 1984. Adapted from Butchko *et al.* (1996).

peak in 1985 and further stated, “In summary, the number of adverse reaction complaints received by the FDA and the nature of these reports in terms of demographic distribution, severity, strength of association with the product, and symptoms remain comparable to those from previous analyses” (FDA, 1995).

FDA also separately analyzed 251 reports of seizure anecdotally associated with aspartame consumption that had been received through ARMS from 1986–1990. FDA concluded that approximately half were highly unlikely to be related to aspartame (Tollefson and Barnard, 1992). Furthermore, the FDA could not exclude the possibility that the remaining reports had not simply occurred by chance. FDA concluded that the anecdotal reports “did not support the claim that the occurrences of the seizures were linked to consumption of aspartame” (Tollefson and Barnard, 1992). It was further concluded that the data did not suggest the need for a controlled clinical study to evaluate this issue. In a subsequent analysis (Tollefson, 1993) of the 265 reports of seizures received from January 1986 through October

1992, FDA concluded that “the anecdotal case reports of seizures received by the FDA do not meet most of the criteria for causality.”

The NutraSweet Company System for Health Report Evaluation

The NutraSweet Company’s postmarketing surveillance system, which continued for 12 years after marketing in the United States, was a collaborative effort between the Consumer Center, where the staff was responsible for data collection, documentation, and follow-up, and the Clinical Research Group, where physicians provided medical expertise for evaluation of the reports. Reports were largely received directly from consumers via telephone or letter. As noted in the CDC and FDA reports, symptoms allegedly associated with aspartame tended to be mild and were also ones that were common in the general population (Butchko and Kotsonis, 1989, 1994, 1996; Butchko *et al.*, 1994, 2001; Butchko and Stargel, 2001, 2002).

As expected, the negative media stories and resulting controversy about aspartame in the early to mid-1980s had a significant impact on the number of anecdotal reports (Fig. 1). The number of reports increased markedly during that time, and, as the controversy decreased in the late 1980s and early 1990s, the number of reports declined while consumption of aspartame did not (Fig. 2). In addition, there was a striking relationship between media reports and the types of reports from consumers (Butchko *et al.*, 1996; Butchko and Stargel, 2001).

As there are more than 100 million aspartame users in the United States alone, it is inevitable that some of them will experience medical ailments temporally associated with consumption of an aspartame-containing product simply by chance. A temporal association does not necessarily imply a causal relationship.

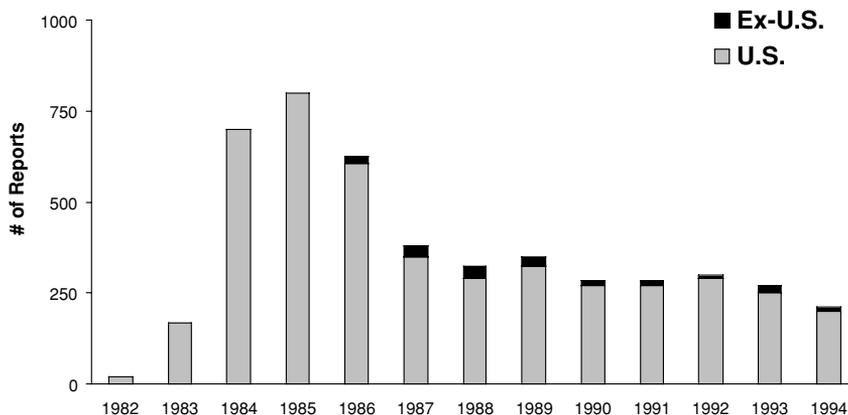


FIG. 2. Anecdotal reports of health effects thought to be related to aspartame peaked in the mid-1980s, shortly after widespread aspartame marketing and declined in the late 1980s. The vast majority of the reports were received from U.S. consumers. Adapted from Butchko *et al.* (1996).

Other Reports

Roberts (1988a,b) reported his analysis of over 500 "aspartame reactors." Most of these individuals were identified by anti-aspartame activist groups. Some were interviewed by Roberts, and almost 400 detailed their symptoms through a nine-page questionnaire. Roberts detailed a myriad of symptoms, which he attributed to aspartame, and suggested that such individuals should be observed for possible aspartame-related symptoms, "preferably before ordering costly tests and multiple consultations." It appears to be a highly unusual practice for a physician to suggest delaying proper diagnosis and treatment of potentially serious symptoms and diseases, based on his personal opinions of patient histories. This work was criticized by Schiffman (1989) as Roberts appeared to ignore the numerous controlled studies that had been published on aspartame safety.

Roberts (1990) also discussed his opinions and anecdotes regarding the cases of health effects he attributed to aspartame in a book that he published. This book was reviewed in the *New England Journal of Medicine* by Rolla (1990), who concluded, "the results are predictably biased, because of the counter-placebo effect, but he presents them as scientific evidence." Rolla continued that Roberts "did not apply a rigid scientific method to test his hypothesis but presents it as fact to the general public without previous scrutiny by his peers. He quotes the *Wall Street Journal* and other newspapers as often as the scientific press." In concluding, Rolla stated, "I appreciate the concern and effort of the author, but my reaction to his book is as negative as it is strong. There is no place for a publication such as this one. It only adds to public misinformation, confusion, and mistrust."

Internet Rumors of Adverse Health Effects

In the past few years, there has been a marked increase in the number of articles and personal testimonials posted on various anti-aspartame Web sites on the Internet alleging that aspartame is responsible for a wide-ranging list of symptoms and serious diseases, including multiple sclerosis, lupus erythematosus, Gulf War Syndrome, chronic fatigue syndrome, brain tumors, and diabetes mellitus. With the widespread dissemination of this alarming information to consumers all over the world, several governments and scientific organizations have addressed these allegations for the public. For example, the Multiple Sclerosis Foundation (Squillacote 1999a,b) evaluated the data on aspartame and concluded that there is no association of aspartame with multiple sclerosis. The medical advisor for this organization further stated, "This campaign by the 'aspartame activists' is not innocent drum banging" as they have created a danger for some individuals who

need appropriate medical treatment for their problems rather than blaming aspartame. Regulatory agencies in several countries, e.g., the United Kingdom (FSA, 2000; Caseley and Dixon, 2001), the United States (Henkel, 1999), and Brazil (Agencia Saude, 1999), evaluated the allegations about aspartame on the Internet and concluded that the information is anecdotal and that there is no reliable scientific evidence that shows that aspartame might be responsible for any of these conditions.

Conclusion

The postmarketing surveillance of anecdotal reports of adverse health effects possibly associated with aspartame was the first such evaluation for a food additive. The extensive monitoring and evaluation of these reports over many years led to the conclusion by epidemiologists that the reported symptoms generally were mild and common in the general population. There was no suggestion of a causal relationship with aspartame. However, "focused" clinical studies would be the best way to address the issues raised by the anecdotal reports. The results of these "focused" studies are discussed in other sections of this review.

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Evaluation of Aspartame and Headaches

Introduction

Headaches are one of the most common human ailments. Although in the majority of cases the symptoms are more uncomfortable than disabling, severe headaches can seriously impair the ability to carry on normal activities. At ambulatory care centers in the United States, headache is the seventh most common complaint, accounting for about 18 million visits per year (Linet *et al.*, 1989). The majority of headache sufferers, however, do not seek medical treatment, especially those from low-income groups, those whose headaches can be treated easily with nonprescription drugs, and those whose headaches are relatively mild and short in duration rather than disabling or long-lasting. Thus the incidence of headache is probably greatly underreported.

Anecdotal Reports of Headache and Aspartame

After aspartame marketing, there were anecdotal reports from some consumers that aspartame may be associated with a variety of health effects. These complaints were evaluated by the Centers for Disease Control (CDC) (CDC, 1984; Bradstock *et al.*, 1986), as well as later by the U.S. Food and Drug Administration (FDA) (Tollefson, 1988; Tollefson *et al.*, 1988). Headache accounted for about 20% of all symptoms reported. Neither CDC nor FDA was able to identify a specific set of symptoms associated with aspartame use that would constitute a public health hazard. Most of the symptoms reported were mild and were also common in the general population. However, they emphasized that carefully designed, focused clinical trials would be the way to determine definitely if there was a causal link between aspartame consumption and any specific adverse effect.

Case Reports and Reports of Single-Subject Tests

Johns (1986) reported the case of a woman with a history of childhood migraine headaches, which recurred at age 31 when she was consuming various foods and beverages sweetened with aspartame. Her headaches reportedly disappeared when she stopped consuming aspartame. When she was rechallenged with aspartame in solution, she developed a migraine headache within 1.5 h but had no headaches after trials with saccharin or sucrose. However, as aspartame and saccharin are easily distinguished by taste, the study was not truly blinded and thereby open to suggestive biases (Gauil, 1986). More recently, Blumenthal and Vance (1997) reported three cases of individuals with long histories of migraine headaches, who self-reported that their headaches worsened when they chewed gum containing aspartame. However, other confounding factors that

may cause headache or the act of chewing itself cannot be ruled out as causative factors. In a letter to the editor, Watts (1991) discussed his headaches, which he felt were related to aspartame.

A single-subject ($N=1$) study with multiple crossovers was conducted by Frasca and Aldag (1988) to determine whether aspartame use was a factor in increased headache frequency experienced by a 27-year-old woman who had also started using oral contraceptives. When both aspartame and the oral contraceptive were discontinued, headache frequency diminished. To determine if aspartame played a role in her headaches, a double-blind, single-patient study was done, which involved four 2-week randomized crossover periods of challenge with aspartame and placebo with a 1-week washout period between each 2-week trial period. No significant difference was found in frequency, duration, or severity of headache between the aspartame and placebo periods.

Questionnaire Evaluations

Lipton *et al.* (1989) distributed a questionnaire to patients seen at a headache clinic to determine their opinions about whether three dietary factors—alcohol, aspartame, and carbohydrates—triggered their headaches. Carbohydrates were included as a negative control because they are believed not to precipitate headaches. Of the 171 persons who completed the questionnaire, 49.7% reported alcohol, 8.2% reported aspartame, and 2.3% reported carbohydrates as possible headache precipitants. They concluded that aspartame may be a dietary trigger of headache in some people. A questionnaire, however, cannot establish a link between aspartame consumption and headache; it only reflects consumers' opinions, which may be biased by the extensive media reports and widespread speculation regarding aspartame that happened at the same time.

Outpatient Studies

Koehler and Glaros (1988) did a randomized, double-blind, placebo-controlled, crossover study in an outpatient setting. The 25 subjects selected for the study had histories of classical migraine headaches and suspected that consumption of aspartame affected their migraine headache activity. During the course of the study, 8 of the subjects dropped out. In addition, 6 others were omitted from the final data analysis, 4 because they had begun new medications during the study period and 2 because their records were incomplete. Thus, only 11 of the 25 subjects (44% of the original sample) completed the study.

After the baseline period, the subjects were randomly selected to receive either capsules containing 300 mg of

aspartame or a matching placebo. Of the 11 subjects remaining at the end of the study, six had greater frequency of migraine occurrence during the aspartame consumption phase than during the placebo phase, resulting in a significantly higher mean incidence of migraine after aspartame vs placebo; however, there were no statistically significant differences in the intensity or duration of migraine after aspartame compared with placebo. Further, there were no statistically significant differences between the aspartame and placebo phases in frequency, intensity, duration, and associated symptoms of tension and unclassified headaches. The researchers concluded that aspartame consumption can increase the frequency and may extend the duration of headache in some persons with a history of migraine.

This study was criticized (Schiffman, 1988) because the many factors in the subjects' daily environment (including diet) that could be related to headache incidence were not controlled and, thus, cannot be eliminated as possible alternative explanations for apparent small differences in headache frequency between treatment and placebo groups. Further, data from only 11 of the 25 subjects were reported, and the effects on frequency of headaches can be attributed largely to data from only 2–3 subjects. Furthermore, the high dropout rate (8 subjects) and the necessity of excluding 6 other subjects from analysis may have biased the representativeness of the remaining eleven (Schiffman, 1988; Amery, 1988). Finally, the statistical tests used to compare mean headache frequencies, intensities, and durations were inappropriate for a crossover study. Thus, given these and other limitations, no reliable conclusions can be drawn from this study.

In another outpatient study, Van Den Eeden *et al.* (1994) did a randomized, double-blind, crossover study with individuals who had self-identified headaches after using aspartame. Of the 32 people entered into the study, only 18 completed the full protocol, 7 completed part of the protocol before withdrawing due to adverse experiences, 3 withdrew for other reasons, 2 were lost to follow-up, 1 was withdrawn for noncompliance with the study protocol, and 1 withdrew with no reason given.

This study consisted of a 1-week baseline period and four 1-week periods—two each with aspartame (30 mg/kg body wt/day) and two with placebo, with a washout day between treatment periods. Prior to the study, subjects were asked whether they were “very sure,” “somewhat sure,” or “not sure” if aspartame was associated with their headaches. The authors reported that there was a statistically significant difference ($P = 0.04$) in the number of days with headache after aspartame compared to placebo. In addition, subjects who were “very sure” that aspartame was associated with their headache prior to the study had statistically greater number of days with headaches after aspartame compared to placebo ($P < 0.001$), whereas there was no statistically significant differences in the

“somewhat sure” and “not sure” groups. There were no significant differences in the duration or intensity of headaches or other adverse experiences after aspartame compared to placebo. From these results, the authors concluded that their study provided evidence of a subset of individuals among those who attributed their headaches to aspartame that are particularly susceptible to aspartame.

Roberts (1995) felt that this study confirmed his case reports of headache, which he attributes to aspartame. However, the study was criticized on several grounds by Levy *et al.* (1995) and Schiffman (1995). Schiffman (1995) discussed that the study, as reported in the journal, appeared to have been a post hoc analysis of the principle author's dissertation, which had not shown any difference between aspartame and placebo in the mean occurrence of headache in all subjects ($P = 0.19$) or among those who were “very sure” that aspartame had caused their headaches ($P = 0.11$). In the new analysis reported in the published article, the authors also changed the way that headaches were counted—in the dissertation, they evaluated mean number of headaches whereas in the paper, they evaluated the number of days that headache occurred. However, this requires an assumption that all days with headache are independent of the other days, when it is well known that headaches often occur in “clusters.”

In addition, the results from only one subject of the 32 enrolled largely accounted for any difference between aspartame and placebo. Levy *et al.* (1995) reanalyzed the data in the Van Den Eeden *et al.* study and concluded that the study results were largely due to the results of one “outlier” subject. Subject 25 reported headaches on 13 of the days on aspartame but on only 2 of the 21 placebo days. Using the methodology of van Den Eeden and co-workers, but with this “outlier” excluded, the odds ratios were not statistically different between aspartame and placebo. When the data were reanalyzed using a random effects logistic regression model, Levy *et al.* found no statistically significant difference between aspartame and placebo in all subjects or those who were “very sure” that aspartame caused their headaches.

Study in a Clinical Research Unit

To evaluate whether aspartame was associated with headache under controlled conditions, Schiffman and colleagues (1987) conducted a clinical study using subjects who had complained to the FDA or to The NutraSweet Company that they were convinced that aspartame had caused their headaches within 24 h after consuming one or more products containing aspartame. The subjects were studied as inpatients at the Duke University Medical Center in Durham, North Carolina, over a 6-day period, allowing close and detailed monitoring of their status as well as elimination of variables,

such as diet, schedule, and activity, which may also impact headaches.

The experimental design was a randomized, double-blind, placebo-controlled, crossover study. On the third and fifth days of hospitalization, each subject was given either aspartame (three doses totaling 30 mg/kg body wt) or a placebo in capsules. For an adult, this total daily dosage would be equivalent to the amount of aspartame contained in about 4 liters of a beverage sweetened 100% with aspartame.

Twenty-six of the 40 subjects experienced headache during the study period. Eight subjects had headache while receiving aspartame but not placebo, whereas 12 had headaches while receiving placebo but not aspartame. Six subjects had headaches with both treatments. Including those 6 subjects, the headache incidence was 35% with aspartame consumption and 45% with placebo. Furthermore, the severity, time between challenge and onset, and duration of the headaches were not statistically significantly different after aspartame vs placebo (Fig. 1). In addition, there were no statistically significant differences in the occurrence of any of the other adverse experiences reported by these subjects when aspartame was compared with placebo. It was concluded that the headache incidence after a large amount of aspartame was equivalent to that after placebo.

This study was criticized by Elsas (1988), Lipton *et al.* (1988), and Steinmetzer and Kunkel (1988). First, it was asserted that studying subjects in a controlled environment rather than their natural environment may have affected the results of the study. However, the only way to control for other variables, which may affect headache, is in such a controlled environment (Schiffman *et al.*, 1988). In fact, the inability to control extraneous factors has compromised other studies evaluating the role of aspartame in headache.

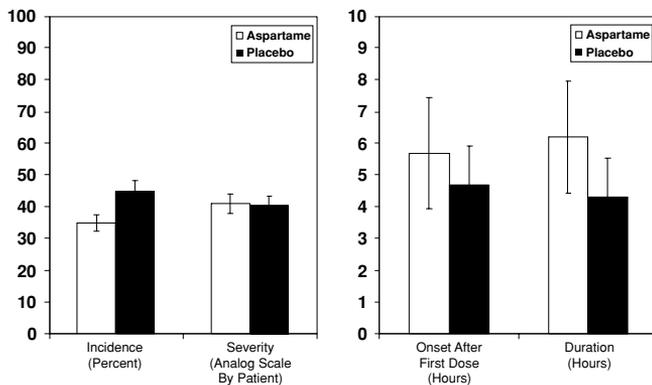


FIG. 1. Mean (\pm SEM) headache incidence (percentage), severity, time of onset after the first dose, and duration after aspartame compared to placebo in 40 subjects who had reported headache associated aspartame. Source: Schiffman *et al.* (1987).

Another comment was that administering the treatments and measuring results over a 24-h period with only a 24-h washout period between the crossover phases does not allow detection of long-term, cumulative effects or those that require the subject to reach a threshold level through continued exposure to aspartame (Elsas, 1988; Steinmetzer and Kunkel, 1988). However, the alleged phenomenon being tested essentially involves a short-term response, and reactions occurring within 24 h are of primary interest (Schiffman *et al.*, 1988). In addition, the results of pharmacokinetic studies have demonstrated that aspartame is rapidly metabolized to its constituent amino acids and methanol (Stegink, 1987), which enter the metabolic pool (Ranney *et al.*, 1976). Aspartame is not absorbed as an intact molecule and cannot accumulate. Thus, an effect of aspartame on headache because of aspartame accumulation is not plausible.

In addition, Elsas (1988) alleged that there was 50% less absorption of aspartame from capsules (used for blinding purposes) compared to solution. However, there is no significant difference in the extent of phenylalanine from aspartame given in capsules vs solution (Burns *et al.*, 1990). He also commented that the study failed to test a mechanistic hypothesis. However, Schiffman *et al.* (1987) did discuss a possible mechanism as circulating concentrations of catecholamines, not aspartame, were associated with headache.

Long-Term Study

The safety of long-term use of large doses of aspartame, extensively studied before the product was released for marketing, was reconfirmed through a 24-week, randomized, double-blind, placebo-controlled, parallel-group study involving 108 normal, healthy subjects (Leon *et al.*, 1989). Subjects were administered three doses of aspartame or placebo each day with their meals, corresponding to a daily dosage of about 75 mg/kg body wt, which is 1.5 times the acceptable daily intake (ADI) of 50 mg/kg body wt in the United States or about 1.9 times the ADI in the European Union. For an adult, this dosage is equivalent to the daily consumption of about 10 liters of a 100% aspartame-sweetened beverage and about 25 times the average daily aspartame intake (90th percentile) by the U.S. general population (Butchko and Kotsonis, 1991).

The most frequent adverse experience was headache, but the incidence rate in the aspartame group was not significantly different from that in the placebo group. The authors concluded that consumption of this very large dose of aspartame daily for 6 months was not associated with any significant changes in the numerous clinical or laboratory parameters or adverse experiences in healthy adults. In addition, the results of the numerous other human studies done with aspartame

do not indicate any association between aspartame and headache (Tschanz *et al.*, 1996).

Conclusion

The controversy regarding aspartame and headache started shortly after marketing when many of the anecdotal consumer reports claimed to be associated with consumption of aspartame were headaches. Headache was associated with aspartame from a questionnaire, as well as single case reports. As headache is also one of the most common symptoms in the general population, it was not possible to decide with certainty whether aspartame was in fact a cause of headache based on anecdotal information. Thus, several studies were done to evaluate this issue. The results of two outpatient studies appear to indicate an association between aspartame and headache. However, statistical issues with results that rely on only a few subjects prevent drawing any valid conclusions from these studies. Another study, which was done in a clinical research unit with individuals who were convinced that aspartame caused their headaches, allowed control over the confounding cofactors that were present in an outpatient setting and found no difference between the occurrence of headache after aspartame and placebo. Finally, numerous human studies have been done with aspartame, and in none of these studies, including a high-dose, long-term study by Leon *et al.* (1989), was there an association between aspartame and headache in adverse experience profiles. Thus, the weight of the scientific evidence indicates that aspartame does not cause headache.

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Evaluation of Aspartame and Seizures and Electroencephalograms (EEGs)

Introduction

Case reports of seizures anecdotally associated with aspartame consumption (Wurtman, 1985; Walton, 1986; Roberts, 1988; Eshel and Sarova-Pinhas, 1993) stimulated interest regarding whether aspartame may potentially be associated with seizures. In addition, anecdotal reports of seizures, which some consumers related to their consumption of aspartame, were reported through passive surveillance and evaluated by the U.S. FDA (Tollefson, 1988) and the Centers for Disease Control (CDC, 1984; Bradstock *et al.*, 1986). Tollefson and Barnard (1992) from FDA also specifically analyzed the anecdotal reports on seizures associated with aspartame received from January 1986 through December 1990. For approximately one-half (49%) of the 251 reports received, it was considered highly unlikely that there was any association with aspartame. Many of the remaining cases had contributing factors (e.g., underlying or concurrent disease or medications) which have been previously associated with seizures. The authors pointed out that only double-blind controlled studies could definitely determine causality. However, based on a review of the surveillance data for aspartame, they concluded that the findings did not merit a scientific study on the association between aspartame and seizures in humans. In a subsequent analysis (Tollefson, 1993) of reports from January 1986 through October 1992, there were then 265 reports of seizure which some consumers related to their consumption of aspartame. Again, FDA concluded, "the anecdotal case reports of seizures received by FDA do not meet the criteria for causality."

Nonetheless, given the seriousness of the claims of seizure associated with aspartame, numerous studies were completed to evaluate whether aspartame or its phenylalanine component has an effect on seizure susceptibility in various animal models of seizure. In addition, several studies in humans have been completed.

Studies in Infant Monkeys

Early studies had reported that infant monkeys fed a diet containing 3000 mg/kg body wt phenylalanine/day experienced grand mal seizures and lasting intellectual deficits. To evaluate whether the phenylalanine provided by aspartame could cause such effects, a pilot study with seven newborn rhesus monkeys was initiated (Rao *et al.*, 1972); unfortunately, only incomplete data are available from this study due to the untimely death of the principal investigator, H. A. Waisman. Based on incomplete data with doses ranging up to about 3600 mg/kg body wt/day, it was noted that some monkeys experienced seizures. However, there were several defects in the study that may account for these

findings (Reynolds *et al.*, 1984). There were too few monkeys in the study, one monkey had severe birth defects, and all animals in the mid- and high-dose groups contracted *Shigella* infections at various times during the study. Further, there was uncertainty if the monkeys had *ad libitum* access to water, which raised the question whether hyperosmolality or generalized hyperaminoacidemia from dehydration may have caused the seizures rather than exposure to phenylalanine. To address this uncertainty, another study was done by Reynolds *et al.* (1984) with 20 infant monkeys given up to 3000 mg/kg body wt/day aspartame or 1650 mg/kg body wt/day of phenylalanine or control added to the diet for 9 months. There were no clinical seizures observed, and there was no evidence of epileptiform activity on EEGs performed at the start of the study and at 4 and 9 months. After completion of this study, these same monkeys were evaluated through learning tests, including measures of object discrimination, pattern discrimination, and oddity learning (Suomi, 1984). Overall, the results did not provide any evidence of either deficits in learning performance or hearing capabilities in these young macaque monkeys.

Studies in Animal Seizure Models

Aspartame was evaluated in several established animal models of seizure. The studies of mice and rats examined the effects of phenylalanine or aspartame on audiogenic seizures, electroshock seizures, chemically induced seizures, and kindling. In light of the hypothesis that aspartame may promote seizures due to an effect on central neurotransmission, the studies done in genetically epilepsy-prone rats (GEPRs) are especially relevant in evaluating whether aspartame causes seizures because seizures in these animals are related to deficits in central noradrenergic and serotonergic neurotransmitter systems (Dailey *et al.*, 1991). Further, photically induced myoclonus studied in the epileptic baboons is similar from an electrophysiological standpoint to generalized seizures in humans. Seizures in these animals also may be affected by changes in brain monoamine concentrations (Meldrum *et al.*, 1989).

Chemically induced models. Pinto and Maher (1988) administered aspartame doses of 200, 500, 1000, and 2000 mg/kg body wt to nonepileptic mice 1 h prior to pentylenetetrazol (PTZ) treatment and noted a significant increase in the number of mice convulsing with the 1000 and 2000 mg/kg body wt aspartame doses. The same response was noted with administration of equimolar amounts of phenylalanine. Guiso and co-workers (1988; Garattini *et al.*, 1988) observed an increase in PTZ-induced convulsions in rats with doses of 750 and 1000 mg/kg body wt aspartame and 250 and

500 mg/kg body wt phenylalanine administered 1 h before PTZ. As a follow-up to their work with PTZ in rats, Guiso *et al.* (1991) reported that tyrosine (1000 mg/kg body wt) protected against the potentiation by aspartame on lowering seizure threshold in PTZ-treated rats. Another study from the same laboratory (Diomedea *et al.*, 1991) demonstrated interspecies differences in the susceptibility to PTZ-induced seizures in animals given aspartame. They evaluated two strains of mice, one strain of guinea pig, and one strain of rat. At doses up to 2000 mg/kg body wt given orally to mice and via the ip route in guinea pigs, there were no significant effects; however, effects were seen in rats.

In another study in rats, Tilson *et al.* (1989) found no effect of 1000 mg/kg body wt aspartame on PTZ-induced seizures. Likewise, in a study in mice using doses of aspartame up to 500 mg/kg body wt at pretreatment intervals of 0.5, 1, and 2 h, there was no effect of aspartame on seizure threshold after PTZ (Nevins *et al.*, 1987). Further, a study with PTZ-induced seizures in the CD-1 mouse (Dailey *et al.*, 1989), using even larger doses of aspartame than used in other studies (up to 2500 mg/kg), demonstrated no effect of aspartame on the convulsive dose in 50% of the animals (CD₅₀) of PTZ. This same laboratory (Dailey *et al.*, 1988) reported in abstract form no effect of aspartame on PTZ-induced seizures in GEPRs. More recently, Helali *et al.* (1996) reported that 1000 mg/kg body wt aspartame given orally did not alter PTZ-induced seizures in mice. However, subacute oral dosing (100 mg/kg body wt/day for 14 days) apparently increased the PD₅₀ (median protective dose) for ethosuximide, sodium valproate, and phenytoin given ip, a condition of questionable relevance to oral dosing of these drugs in humans.

It is interesting that, although an acute dose of 750–1000 mg/kg body wt aspartame on PTZ-induced seizures in rats significantly increased the number of rats having tonic-clonic seizures (Garattini *et al.*, 1988; Guiso *et al.*, 1988), these authors found no such effect when the dose of aspartame (1000 mg/kg body wt) was given in three divided doses over 2 h, after a meal, or ingested overnight with food and drinking water (2000 mg/kg body wt). This led Garattini and co-workers to conclude, "All these data taken together indicate that the potentiation of metrazol [pentylenetetrazol] convulsions by aspartame has no relevance to the practical use of aspartame even at "abuse" doses."

Pinto and Maher (1988) reported that aspartame (1000–2000 mg/kg) and an equimolar amount of phenylalanine (560 mg/kg body wt) decreased latency to fluorothyl-induced convulsions in CD-1 mice. In contrast, Sperber *et al.* (1995) demonstrated no effect of *in utero* exposure to aspartame (either 500 or 750 mg/kg body wt/day) in guinea pigs. At 30 days of age, offspring were exposed to fluorothyl. Compared to a nontreated control group, there were no statistically significant differences in either clonic or tonic seizures in either of

the aspartame dose groups. The authors concluded that *in utero* exposure to aspartame has no epileptogenic potential in the offspring in the fluorothyl model.

Chin and Woodbury (1988) reported in abstract form an effect of aspartame on bicuculline-induced seizures in DBA/2 mice; however, a subsequent study also reported in abstract form did not confirm effects of aspartame with bicuculline-induced seizures in DBA/2 mice (Bettendorf *et al.*, 1989). Further, no effects of oral aspartame administration were observed on quinolinic acid-induced seizures in rats (Guiso *et al.*, 1988) or on theophylline (Zhi and Levy, 1989) and lidocaine seizure models in CD-1 mice (Kim and Kim, 1987; Kim *et al.*, 1988).

Electroshock-induced seizures. With the exception of one report in mice in abstract form (Chin and Woodbury, 1988), there was no effect of aspartame in the electroshock paradigm in mice reported by other investigators (Nevins *et al.*, 1987) and in two strains of GEPRs with doses up to 3000 mg/kg body wt (Jobe *et al.*, 1992). In addition, experiments in Sprague–Dawley rats (Guiso *et al.*, 1988) with doses of aspartame of 1000 mg/kg body wt failed to modify tonic hindlimb extension induced by electroshock. Tilson *et al.* (1989) evaluated the effect of 1000 mg/kg body wt of aspartame on electroshock-induced seizures in Fischer 344 rats and found no effect on the number of animals developing tonic seizures after electroshock.

Kindling. Tilson *et al.* (1989) also evaluated the effect of aspartame (1000 mg/kg body wt) on the rate of kindled seizures in which one group of rat pups were dosed on days 3–13 (neonatal) and another group were dosed on days 21–35 (postweaning). The rate of kindling was tested in both groups of rats at 90 days of age. There were no effects of aspartame on kindled seizures in either group. In another study to evaluate aspartame and kindled seizures, Cain *et al.* (1989) gave rats up to 200 mg/kg body wt aspartame in the group evaluated with amygdala-kindled seizures (the amygdala has the lowest afterdischarge threshold of any structure in rat brain) and up to 2000 mg/kg body wt aspartame in the group with hippocampal-kindled seizures. Despite these large doses of aspartame, there was no effect on afterdischarge threshold or seizure strength.

Seizures in susceptible animal strains. Dietary phenylalanine was reported over 25 years ago to facilitate audiogenic seizures in DBA/2 mice (Schlesinger *et al.*, 1969; Truscott, 1975), and subsequently it was reported in abstract form that aspartame increases audiogenic seizure intensity in DBA/2 mice (Chin and Woodbury, 1988). Subsequent studies, however, have found no effect of aspartame (at doses up to 2000 mg/kg body wt) on audiogenic seizures in DBA/2 mice (Jobe *et al.*, 1989a,b). Also, in a study with two strains of

GEPRs, Dailey and colleagues (1991) found no effects of aspartame on audiogenic seizures after acute doses up to 2000 mg/kg body wt and doses averaging 863 mg/kg body wt/day for 28 days. This lack of an effect on seizures was noted despite marked increases in Phe/LNAA after acute doses of 1000 and 2000 mg/kg body wt aspartame. In addition, the effect of doses of aspartame up to 1000 mg/kg body wt on seizure susceptibility and severity in light-induced myoclonus was studied in baboons which show photosensitive responses similar to generalized seizures in humans (Meldrum *et al.*, 1989). Despite up to 34-fold increases in Phe/LNAA in these baboons, there was no effect of aspartame on seizures.

Studies in Humans

Double-blind studies of the effects of aspartame on seizures and EEGs have been done in humans (Anderson *et al.*, 1996). These include the study by Rowan and colleagues (1995) on seizures and EEG activity in adults and children who had reported seizures after aspartame consumption, the study by Camfield and colleagues on EEG activity in children with absence seizures (Camfield *et al.*, 1992), and the study of Shaywitz and colleagues (1994) on seizure and EEG activity in epileptic children. In addition, EEGs have been evaluated after large doses of aspartame in both PKU heterozygotes (PKUH) (Trefz *et al.*, 1994) and normal individuals (Spiers *et al.*, 1998).

Camfield and colleagues (1992) studied EEG spike-wave activity in 10 children with newly diagnosed, untreated, absence seizures. On consecutive days, children received 40 mg/kg body wt aspartame or 1.6 g/kg body wt sucrose in 250 ml of orange juice in a randomized, double-blind, and placebo-controlled study. On comparison of aspartame and sucrose treatment periods on 24-h EEG cassette recordings, no statistically significant differences were seen for the number of spike-wave bursts or the mean length of spike-wave discharges. However, a statistically significantly greater total duration of spike-wave activity/hour (increase of $40 \pm 17\%$; $P = 0.028$) was reported in the aspartame group. The authors concluded that aspartame appeared to increase the amount of EEG spike-wave activity in children with absence seizures. However, Shaywitz and Novotny (1993) criticized this study because the use of sucrose as the placebo may have confounded the results of the study as glucose has been reported to affect EEG activity. Further, it is unclear whether spike-wave activity was similar in the baseline periods that preceded aspartame and sucrose. Although it was reported that there was no significant difference in spike-wave activity during the two baseline periods, the P value given for the comparison of baseline periods ($P = 0.203$) suggests that a fairly substantial difference did exist for the pre-aspartame vs pre-sucrose baseline activity. In addition, because of the high variability of spike-wave

TABLE 1
Seizure History in Children with Seizure Disorders Studied with Aspartame and Placebo^a

Subject	Age (years)	Seizure type	Duration (years)
1	8	Complex partial	6
2	5	Generalized tonic-clonic and complex partial	3
3	8	Generalized and absence	1
4	12	Generalized tonic and atypical absence	3
5	11	Generalized motor	5
6	13	Absence	Unknown
7	10	Generalized motor	4
8	8	Generalized tonic and absence	1
9	7	Generalized motor and absence episodes	1
10	10	Complex partial	1

^a Adapted from Shaywitz *et al.* (1994).

discharge occurrence in children with absence seizures, a longer baseline period than used by the authors is necessary to determine whether any apparent changes were actually outside the range of normal variability.

Shaywitz and colleagues (1994) evaluated the effect of aspartame (34 mg/kg body wt/day) compared with placebo (2 weeks on each treatment) on EEG activity and seizures in 10 children with seizure disorders, including one child with absence seizures (Table 1). The study was a randomized, double-blind, placebo-controlled, crossover study. It was hypothesized that children with a history of seizures would represent a particularly susceptible population, not only because of a history of seizures but also because children have the highest intake of aspartame on a milligram per kilogram of body weight basis (Butchko and Kotsonis, 1991). The biochemical and neurochemical effects of aspartame consumption in plasma were also evaluated. Plasma concentrations of phenylalanine, other large neutral amino acids, methanol, and formate, as well as blood and urine concentrations of monoamine neurotransmitters and their metabolites, were measured.

Nine of the 10 children who enrolled in the study completed both treatment arms. One subject dropped out following the first arm (placebo) for non-study-related reasons. Despite statistically significant increases in plasma phenylalanine concentrations and the Phe/LNAA ratio, no exacerbation of seizures was noted. No clinical or electroencephalographic seizures were noted, and there were no statistically significant EEG differences between treatments. The findings from the standard and 24-h EEGs were similar during aspartame and placebo arms for 7 of the 9 children who completed both treatment arms (Tables 2 and 3). One child had a more abnormal EEG tracing on the standard 21-lead EEG during the placebo period (first treatment),

TABLE 2

Clinical Evaluation of Standard 21-Lead EEGs in Children with Seizure Disorders after Aspartame and Placebo^a

Subject	Aspartame	Placebo
1	Normal	Normal
2	Normal	Normal
3	Mildly abnormal	Mildly abnormal
4	Very abnormal	Very abnormal
5	— ^b	Normal
6	Mildly abnormal	Mildly abnormal
7	Mildly abnormal	Moderately abnormal
8	Moderately abnormal	Moderately abnormal
9	Normal	Normal
10	Moderately abnormal	Moderately abnormal

^a Adapted from Shaywitz *et al.* (1994).

^b Subject 5 withdrew from the study for personal reasons after the first treatment arm (placebo) and thus did not participate in the aspartame treatment arm.

and another subject had a more abnormal 24-h EEG during the placebo period compared with the aspartame period. There were also no statistically significant differences in behavior ratings by either parents or teachers between the aspartame and placebo treatments and no statistically significant differences in adverse experiences between placebo and aspartame treatments.

Rowan and co-workers (1995) evaluated the effect of 50 mg/kg body wt aspartame compared with placebo on seizure incidence and EEG epileptiform discharges in a randomized, double-blind, placebo-controlled,

TABLE 3

Clinical Evaluation of 24-h Cassette EEGs in Children with Seizure Disorders after Aspartame and Placebo^a

Subject	Aspartame	Placebo
1	Normal	Normal
2	Normal	Normal
3	Normal	Normal
4	Abnormal: bursts of generalized sharp waves, Gibbs pattern increased during sleep	Abnormal: bursts of generalized sharp waves and occasional slow-spike, slow-wave discharges
5	— ^b	Normal
6	Normal	Normal
7	Abnormal: right hemisphere epileptiform activity, increased in sleep	Abnormal: right hemisphere epileptiform activity increased in sleep
8	Abnormal: background abnormality	Abnormal: generalized spike and slow-wave activity
9	Normal	Normal
10	Abnormal: generalized spike and slow-wave activity	Abnormal: interictal epileptiform activity

^a Adapted from Shaywitz *et al.* (1994).

^b Subject 5 withdrew from the study for personal reasons after the first treatment arm (placebo) and thus did not participate in the aspartame treatment arm.

crossover study. Eighteen subjects (16 adults and 2 children) who had experienced seizures purportedly as a result of aspartame consumption were evaluated in clinical research units with 5 days of continuous EEG monitoring. The subjects had a variety of types of seizures, including 4 with a history of absence seizures. During the study, there were no clinical seizures reported in either treatment arm. However, two subjects did experience electrical seizures noted on the EEG during the placebo (first treatment) arm of the study. Quantification of the EEG epileptiform activity during the study revealed no difference between aspartame and placebo. In addition, EEG recordings were also rated for sleep stage. There were no statistically significant differences with aspartame compared to placebo treatment for any of the sleep variables assessed, i.e., total sleep time, total time awake, percentage of time awake, and percentage of time in sleep stages I–IV and REM. Thus, it was concluded that aspartame was no more likely to cause seizures than placebo and that there was no evidence of aspartame-activated epileptiform activity on the EEG or effects on sleep.

In response to suggestions from one laboratory that phenylalanine decreases the mean power frequency of EEGs in a small number of PKUH individuals (Elsas and Trotter, 1988; Epstein *et al.*, 1989), Trefz and colleagues (1994) evaluated EEGs in 48 PKUH individuals after a baseline period followed by aspartame (either 15 or 45 mg/kg body wt/day) and placebo for 12 weeks on each treatment in a randomized, double-blind, crossover study. However, no statistically significant differences were seen between the aspartame and placebo treatments on clinical evaluation of the EEG or on detailed inspection of the EEG spectral parameters studied. In another study, Spiers *et al.* (1998) evaluated EEGs in healthy adults after large daily doses of aspartame (15 or 45 mg/kg body wt/day) vs sugar for 20 days on each treatment compared to a 4-week baseline period. In addition, there was a 1-week washout between treatments. There were no EEG abnormalities associated with aspartame.

Conclusion

Numerous studies using various animal epilepsy models and several studies in humans have been done to evaluate whether aspartame has an effect on seizures or EEGs (reviewed by Jobe and Dailey, 1993). The studies in animals used doses of aspartame up to 3000 mg/kg body wt or 1000 times the 90th percentile human intake in the United States of 3.0 mg/kg body wt/day (Butchko and Kotsonis, 1991; Butchko and Stargel, 2001). With few exceptions, specifically some studies of PTZ-induced seizures in rats after very large doses of aspartame, which were not reproducible in other laboratories, most of the published studies have shown no effects of aspartame on seizure susceptibility. In the case of the PTZ

model, any effects appear to be largely situational and species specific as no effects were seen when the dosage was given as divided doses, after a meal, or ingested overnight with food and water. There was also no effect of aspartame on seizures in nonhuman primates.

In addition to the animal studies, several studies were done in humans. The study by Camfield and co-workers (1992) suggested an effect of aspartame in children with absence seizures on the time per hour spent in spike-wave discharges. However, the study lacked a true placebo, as the sucrose used as a placebo could have itself confounded the results of the study. Further, the study had serious methodological issues because of the inadequate length of baseline period for EEG activity. Individuals who were convinced that aspartame was responsible for their seizures and children with seizure disorders were also evaluated. The results of these studies, using doses of aspartame more than 10 times the 90th percentile average daily aspartame intake in the United States, along with the results of other studies in healthy individuals and PKUH, demonstrated no effects of aspartame on clinical seizures or EEGs. Thus, when considering the totality of the data, the evidence is clear that aspartame, even in amounts greatly exceeding the 90th percentile intake, is not a proconvulsant and does not affect seizure susceptibility.

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Evaluation of Aspartame and Behavior, Cognitive Function, and Mood

Introduction

It was suggested that aspartame might increase the ratio (Phe/LNAA) of phenylalanine (Phe) to the other large neutral amino acids (LNAA) that compete for entry into the brain and that, as such, aspartame might affect central neurotransmitter concentrations (see Phenylalanine and Neurochemistry section). It was further suggested that this, in turn, might affect brain function, leading to changes in behavior, cognitive function, or mood. Although no behavioral effects had been noted in animals in the preapproval toxicology testing, a number of studies in animals, using various testing paradigms, were subsequently done to evaluate behavior specifically. In addition, studies have been done in healthy adults, PKU heterozygous adults (PKUH), healthy children, and children with attention deficit disorder (ADD) or “hyperactivity” (ADHD) or in children who were thought to be “sugar sensitive.”

Studies with Animals

In early studies, several groups examined the effects of aspartame and phenylalanine on animal behavior. In studies of learning performance in young stump-tail macaque monkeys, no effects were seen for doses of aspartame up to 3000 mg/kg body wt given chronically (Suomi, 1984). Learning tests included measures of object discrimination, pattern discrimination, and oddity learning and were given to 20 macaques who had received a control diet, diet with aspartame (1000, 2000, or 3000 mg/kg body wt), or diet with phenylalanine only added (1650 mg/kg body wt) for 270 days beginning in infancy. Overall, the results did not provide any evidence of significant learning performance or hearing differences between these groups of young macaques.

Several other groups have used different models to evaluate whether aspartame has an effect on behavior but some, such as Holder and Yirmiya (1989), used an ip dosing paradigm. This method of administration is not relevant to the dietary intake and oral consumption of aspartame by humans.

Mullenix *et al.* (1991) evaluated spontaneous behavior in rats 1 h after large doses of aspartame (500 and 1000 mg/kg body wt), phenylalanine, and tyrosine using a computerized pattern recognition system that identified and classified 13 different behavioral acts. Motor output was evaluated by the number of behavioral initiations, total time that activity was performed, and a calculation of the behavioral sequence structure. Amphetamine was used as a positive control. Neither aspartame, nor phenylalanine, nor tyrosine induced any changes in spontaneous motor behavior output despite significant changes in Phe/LNAA. The investigators concluded that there was no correlation

between Phe/LNAA and objectively measured behavioral effects.

Tilson *et al.* (1991) evaluated sensorimotor function, learning, and memory in several experimental paradigms in rats. Neither the acute nor repeated (14 days) administration of aspartame by gavage (up to 1000 mg/kg body wt) had a significant effect on routine measures of sensorimotor function, including spontaneous motor activity or acoustic startle reflex and prepulse inhibition. They also found no effect of aspartame (500 or 1000 mg/kg body wt) on acquisition of passive or active avoidance or a spatial, reference memory task in the Morris water maze. These authors concluded that large doses of aspartame have no significant effects in adult rats when measured by procedures known to be sensitive to the neurobiological effects of neurotoxins.

Goerss *et al.* (2000) used the standard resident-intruder paradigm to evaluate whether aspartame (200, 400, and 800 mg/kg body wt given ip) had an effect on aggression in rats. According to the authors, previous studies had demonstrated an inverse relationship between aggression and concentrations of brain serotonin. Goerss *et al.* (2000) similarly noted a significant increase in striatal serotonin concentrations after aspartame that was associated with a decrease in aggressive behaviors in their experimental animals. However, intraperitoneal injection of aspartame used in this study is not relevant to human exposure.

LaBuda and Hale (2000) evaluated whether aspartame interfered with the anxiolytic actions of ethanol, as measured by plus-maze performance of rodents. Aspartame (1000 and 2000 mg/kg body wt) was followed by either a vehicle or ethanol, all given ip. The aspartame had no significant effect on anxiety-related behavior, nor did it interfere with the anxiolytic action of ethanol. However, as noted above, the relevance of ip aspartame dosing in animals is questionable.

Studies with Healthy Human Adults

Ryan-Harshman and co-workers (1987) evaluated the effect of aspartame (5040 and 10,080 mg) and phenylalanine (840, 2520, and 5040 mg) on energy intake and macronutrient selection and on feelings of hunger, mood, and arousal in healthy adult males. Despite significant increases in plasma phenylalanine concentrations and Phe/LNAA (high mean Phe/LNAA was 0.397 after the 10,080-mg dose of aspartame), these authors found no effect of aspartame or phenylalanine on short-term energy and macronutrient intakes or subjective feelings of hunger, mood, or arousal.

In a study to determine whether aspartame affected neuropsychological function in 20 normal adults

(Lieberman *et al.*, 1988), aspartame (60 mg/kg body wt) was compared to or combined with carbohydrate in a blinded single-dose study monitoring mood and performance tasks. The behavioral tests administered were: an auditory reaction time task, a visual reaction time task, the Wilkinson auditory vigilance test, the Digit Symbol Substitution Test (DSST), a tapping test, the profile of mood states (POMS), the visual analogue mood scales (VAMS), and the Stanford sleepiness scale (SSS). Despite increases in Phe/LNAA in the treatment arms with these large doses of aspartame, both with and without carbohydrate, no effects of aspartame on behavior were detected.

Lapierre *et al.* (1990) evaluated 10 subjects who were given either aspartame (15 mg/kg body wt) or placebo in a double-blind, crossover design. Measurements were made on mood with the VAMS, DSST, and a reaction time task. Learning and memory were assessed with a 16-item word list. No adverse effect of aspartame was found despite a demonstrable change in plasma phenylalanine concentrations after aspartame dosing. In another study (Pivonka and Grunewald, 1990), 120 young college women were given placebo, sucrose-sweetened drink, or aspartame-sweetened drink (dosing between 180 and 280 mg). The testing included SSS, VAMS, and POMS. No adverse effects of aspartame on mood were found in this single-dose paradigm.

Aspartame was also tested in 12 airplane pilots at a single dose of 50 mg/kg body wt in a blinded, randomized, placebo-controlled, crossover design study (Stokes *et al.*, 1991). The measurements were through a commercial test, the SPARTANS cognitive battery of aviation-related information processing tasks, including spatial and sequencing abilities, memory, planning, sensory motor function, coordination, response inhibition, and complex mental processes. No effect was noted on the cognitive efficiency of these pilots when aspartame was compared to placebo.

In another study using the same test battery with dosing of 50 mg/kg body wt aspartame and placebo for nine days in 12 healthy adults, aspartame had no effects on SPARTANS performance (Stokes *et al.*, 1994). As might be expected, however, alcohol was associated with a significant decrement in psychomotor skills in these subjects when compared to either placebo or aspartame. Similarly, Dodge *et al.* (1990) reported no effect of aspartame (50 mg/kg body wt) on cognitive function in pilots, either acutely or after 8 days of exposure.

Spiers and colleagues (Spiers *et al.*, 1998; Schomer *et al.*, 1996) evaluated whether acute or repeated administration of aspartame had any neuropsychologic or neurophysiologic effects. This study was a follow-up to a preliminary study these authors had reported earlier (Spiers *et al.*, 1988), which had received widespread media coverage and suggested a possible effect of aspartame. After a 1-month baseline period, 48 subjects participated in a randomized, double-blind, crossover

TABLE 1
Neuropsychologic Functions Tested on Days 10 (1.5 h after Dose) and 20 (before AM Treatment) after Aspartame, Sucrose, and Placebo Treatments^a

Neuropsychologic function	Tests
Verbal learning	20-word list, 5 trials, free recall
Verbal attention span	Digit span (forward and backward)
Spatial attention span	Corsi Block Test (forward and backward)
Short-term memory	Free recall of word list
Verbal fluency	Controlled Oral Word Association
Response set alternation	Trailmaking Tests (form A and form B)
Response set inhibition	Stroop Test, interference condition
Motor response set alternation	Auditory Reciprocal Motor Programs
Motor response set inhibition	Auditory, Go–No-Go
Overall cognitive efficiency	ThinkFast
Long-term memory	Free recall of word list

^a Adapted from Spiers *et al.* (1998).

protocol in which they received, for 1 month each, aspartame (15 mg/kg body wt/day, $N = 24$; or 45 mg/kg body wt/day, $N = 24$), sucrose, and placebo that were consumed in a combination of capsules and solution. On days 10 and 20 of each treatment period, subjects were evaluated for mood and cognitive function (Table 1), and electroencephalograms (EEGs) were evaluated. Testing on day 10 represented an acute condition as testing was administered 1.5 h after the dose, and testing on day 20 represented a chronic condition as testing was done before AM dosing. Despite significant elevations in subjects' Phe/LNAA ratios (Fig. 1), no difference was found between aspartame and the other treatments on the adverse experiences reported, the mood ratings, and the neuropsychological and neurophysiological parameters measured in this study. In addition, there were no EEG abnormalities associated with any treatment. Thus, these findings did not support the authors' initial hypothesis.

Studies with Adults Heterozygous for Phenylketonuria (PKUH)

Individuals who are homozygous for PKU have markedly elevated plasma phenylalanine concentrations. If not treated with a special, low-phenylalanine diet starting at birth, these individuals are subject to a variety of neurologic and cognitive deficits. As a consequence, issues were raised regarding the potential for aspartame to affect cognitive functioning in PKU heterozygotes (PKUH). Trefz *et al.* (1994) did a randomized, double-blind, placebo-controlled, crossover study in 49 PKUH subjects. After a 4-week baseline period, subjects were given either 15 or 45 mg/kg body wt/day aspartame and placebo for 12 weeks on each treatment.

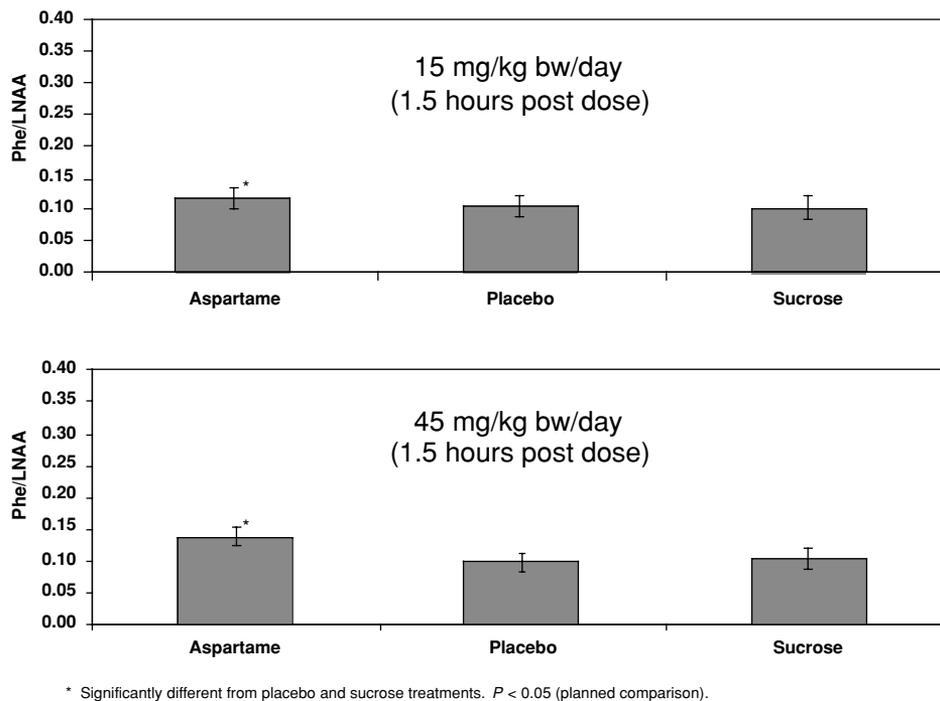


FIG. 1. Phe/LNAA 1.5 h after aspartame, placebo, and sucrose treatments on day 10 in healthy adults. Adapted from Spiers *et al.* (1998).

Subjects were tested with the “Amsterdam Neuropsychological Tasks” battery (de Sonneville, 1999), including tests for visual information processing and executive function of varying complexity. This computerized test battery has demonstrated changes in cognitive performance to be associated with changes of plasma phenylalanine concentrations in individuals with PKU (Schmidt *et al.*, 1994), even in a very well-controlled population (Huijbregts *et al.*, 2002). There was no difference between either dose of aspartame and placebo on this battery of neuropsychological tests.

Studies with Children

Many of the studies in children have examined the behavioral and cognitive effects of aspartame in children with attention deficit disorders (ADD), with “hyperactivity” (ADHD), or with alleged “sugar sensitivity” (Wolraich *et al.*, 1985; Milich and Pelham, 1986; Mahan *et al.*, 1988; Hoover and Milich, 1994; Shaywitz *et al.*, 1994b). A number of studies have evaluated both normal children and children with ADD, ADHD, or “sugar sensitivity” (Ferguson *et al.*, 1986; Kruesi *et al.*, 1987; Wender and Solanto, 1991; Wolraich *et al.*, 1994), while some studies have focused only on normal children (Goldman *et al.*, 1986; Rosen *et al.*, 1988; Saravis *et al.*, 1990). In addition, one study evaluated behavior in children with seizure disorders given aspartame (Shaywitz *et al.*, 1994a). Many of the studies with children were primarily concerned with assessing the effects of sugar while aspartame was used as

a sweet placebo control (Wolraich *et al.*, 1985; Goldman *et al.*, 1986; Milich and Pelham, 1986; Rosen *et al.*, 1988; Mahan *et al.*, 1988; Wender and Solanto, 1991; Hoover and Milich, 1994).

Studies with normal children. In a two-phase study with 20 children aged 9–10 years (Saravis *et al.*, 1990), single doses of aspartame (34 mg/kg body wt) and cyclamate in equivalent sweetness amounts were given with carbohydrate in one phase, and, in the other, aspartame (approximately 10 mg/kg body wt) was compared with sucrose. Measurements included an associative learning task, an arithmetic test, the Children’s Depression Inventory, the State Scale of the State-Trait Anxiety Inventory for Children, and several observational measures. The results suggested that when consumed with carbohydrate, aspartame had no effect on learning, behavior, or mood. The authors reported decreased minor and gross motor behavior following sucrose consumption, as compared with aspartame.

Goldman *et al.* (1986) used a double-blind, crossover design to evaluate the effect of sucrose added to juice on the behavior of eight preschool children. Aspartame was used as the sweet placebo control. A decrement was found in performance on structured testing and increased “inappropriate” behaviors were observed during free play after the children consumed sucrose but not aspartame. Goldman *et al.* (1986) concluded that this study provided objective evidence of an effect of sucrose on behavior. In another double-blind, crossover study in which aspartame was the placebo, Rosen *et al.*

(1988) evaluated the effect of sucrose on behavior in 45 preschool and elementary school children. Only small effects were observed and only after sucrose intake. As such, the authors concluded that these were not clinically significant.

In a study of the effect of sweetness on newborn crying, Barr *et al.* (1999) compared the effects of sucrose, aspartame, polycose (a nonsweet carbohydrate), and water. Relative to water, both sucrose and aspartame reduced crying and transiently increased mouthing and hand–mouth contact. Polycose had no effect on either parameter. The authors concluded that newborns respond preferentially to sweetness rather than to carbohydrate, but there was no difference between sucrose and aspartame.

Studies with ADD, ADHD, or sugar-sensitive children. Children with ADD and ADHD represent especially interesting sample populations. Specifically, it has been proposed that the three major monoamine neurotransmitters, dopamine, norepinephrine, and serotonin, may all play a role in the pathophysiology of these disorders. The possibility that aspartame might affect the biosynthesis of these monoamine neurotransmitters led to speculation that aspartame might cause neurochemically mediated changes in ADD- and ADHD-related behaviors.

Wolraich *et al.* (1985) evaluated sucrose compared to placebo (aspartame) on behavior (playroom observations and examiner ratings) and cognition (learning and memory tasks) in 16 hyperactive boys. They found no effects of sucrose or aspartame on behavior or cognitive function. Milich and Pelham (1986) evaluated the effect of sugar on behavior and academic performance in 16 boys diagnosed with ADD. Aspartame was used as the placebo. They found no effect on classroom behavior, noncompliance with adult requests, and peer interactions and no effects on academic productivity and accuracy.

In another study of 16 children who were described as overly aggressive, loud, and noncompliant after ingesting sugar, Mahan *et al.* (1988) found no significant behavior changes using an open-label paradigm. Five children who had changed from baseline on a few of the parameters tested were then evaluated more closely with sugar, honey, tapioca, and starch in a double-blind challenge that used aspartame as a placebo. No consistent effects were found for any of the challenge substances on either learning or attention span.

To examine the effect of sugar ingestion on mother–child interactions, Hoover and Milich (1994) studied 35 boys, 5 to 7 years old, who were reportedly sugar sensitive. Mothers were told that the children would receive either sugar or placebo (aspartame) but all subjects, in fact, received only aspartame. Mothers who were told that their children were in the experimental, supposedly sugar, group rated their children as being

more hyperactive than those in the placebo (aspartame) group.

Shaywitz *et al.* (1994b) conducted a randomized, double-blind, placebo-controlled, crossover study with aspartame in children diagnosed with ADD. In this study, 15 children with ADD received 34 mg/kg body wt/day of aspartame and placebo for 2 weeks on each treatment. Several measures of behavior and cognitive function were evaluated, including the Matching Familiar Figures Test, Children’s Checking Task, the Airplane Test, the Wisconsin Card Sorting Test, the Multigrade Inventory for Teachers, and the Conners Behavior Rating Scale, as well as the Subjects Treatment Emergent Symptom Scale. There were no clinically significant differences between aspartame compared with placebo on behavior or cognition measures in these potentially sensitive children. In another study in 10 children with seizure disorders, Shaywitz *et al.* (1994a) also evaluated behavior after 2 weeks of aspartame (34 mg/kg body wt/day) compared to 2 weeks of placebo using a modified Connors Behavior Rating Scale. There was no statistically significant difference between aspartame and placebo treatments on the behavior ratings made by either the teachers or the parents.

Studies with both normal and sensitive children. Ferguson *et al.* (1986) evaluated behavior and cognitive function in eight boys and girls ages 5 to 13 years who were believed by their parents to have adverse responses to sugar (five with ADD, one with a neuropsychological disorder, and two with no diagnosable disorder). The study was a randomized, double-blind, crossover design comparing sugar and aspartame. There was no evidence for a sucrose effect vs aspartame and no change from baseline after either sucrose or aspartame. These investigators also used a randomized, double-blind, crossover design to compare activity level (actometer, parental and teacher assessments), behavior, fine and gross motor coordination, and visual–motor coordination in 18 normal preschool boys and girls after sugar vs aspartame. When evaluating the group data, there was no difference between aspartame or sucrose and baseline in behavior, activity level, or fine or gross motor coordination; only after sucrose, was there a difference in comparison to the baseline and aspartame conditions on a developmental drawing task.

Kruesi *et al.* (1987) evaluated aggression and activity after sugar, aspartame, saccharin, and glucose in a double-blind, crossover study in 30 preschool boys—18 considered “sugar reactive” by their parents and 12 healthy boys. There were no effects after any treatment on aggression or observers’ ratings of behavior. There were lower actometer counts after aspartame, which were not verified by observer ratings. The authors concluded that neither aspartame nor sugar is a clinically significant cause of disruptive behavior in children.

In another study of 9 normal children and 17 children with ADD or ADHD, Wender and Solanto (1991) evaluated the effect of sugar in comparison to aspartame and saccharin placebos on attention and aggressive behavior. Children with ADHD were significantly more aggressive than were normal children; however, there were no effects of sugar, aspartame, or saccharin on the incidence of aggressive behaviors. Inattention, however, was greater in the ADHD group after sugar consumption compared to either aspartame or saccharin.

A study of the effects of diets with sucrose, aspartame, and saccharin on behavior and cognitive performance in normal children has also been reported (Wolraich *et al.*, 1994). Twenty-three school-age children who had been reported to respond adversely to sugar and 24 normal preschool children were given each regimen. The children received a different diet for each of three consecutive 3-week periods; one diet was high in sucrose with no high-intensity sweeteners; another diet was low in sugar and contained aspartame; and the other was low in sugar and contained saccharin. Preschool children consumed a mean (\pm SD) of 5600 ± 2100 mg/kg body wt/day sucrose while on the sucrose diet, 38 ± 13 mg/kg body wt/day aspartame while on the aspartame diet, and 12 ± 4.5 mg/kg body wt/day saccharin while on the saccharin diet. The school-age children thought to be sensitive to sugar consumed 4500 ± 1200 mg/kg body wt/day sucrose, 32 ± 8.9 mg/kg body wt/day aspartame, and 9.9 ± 3.9 mg/kg body wt/day of saccharin while on those diets. Behavior and cognitive performance were evaluated weekly. For children described as being sugar sensitive, there was no difference among the three diets on any of 39 behavioral and cognitive parameters. In the normal preschool children, only 4 of the 31 variables differed among the diets, but there was no consistent pattern in the differences. The parent's rating of cognition was significantly better with sucrose than either aspartame or saccharin, and the grooved pegboard performance was significantly slower after sucrose compared to the other sweeteners for both dominant and non-dominant hands and the total score. The authors concluded that neither sucrose nor aspartame in amounts exceeding typical dietary levels predictably affects behavior or cognitive function in children.

Conclusion

Numerous animal studies have been carried out using various testing paradigms and very large doses of aspartame that have sometimes been hundreds of times the 90th percentile intake for humans in the United States. The only effects observed were after ip dosing, a paradigm that is not comparable to the dietary consumption of aspartame in humans. Taken as a whole, and despite significant changes in Phe/LNAA ratios, the findings from the animal research reviewed fail to provide support for what have been purported to be the

adverse effects of aspartame exposure on behavior. Nor have they provided any mechanistic or neurophysiological basis for any such putative effects.

It is perhaps not surprising, therefore, that controlled studies in humans have similarly failed to demonstrate any adverse behavioral or cognitive effects of aspartame. This body of literature has included studies conducted with healthy and potentially vulnerable (PKUH) adults and with healthy and potentially vulnerable children, including children with ADD, ADHD, or alleged "sugar sensitivity," or in children with seizure disorders. Various objective tests, self-rating scales, and naturalistic observations have been utilized in protocols investigating both acute and chronic exposure to high doses of aspartame. Overall, a considerable body of human research has demonstrated that aspartame has no effect on behavior, cognitive function, or mood.

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Evaluation of Aspartame and Allergic-Type Reactions

Introduction

Allergic-dermatologic reactions attributed to aspartame ingestion accounted for about 15% of the anecdotal complaints evaluated by the Centers for Disease Control (CDC, 1984). Included as allergic complaints were rashes, sore throat/mouth, swelling, and itching. In addition to the CDC evaluation, there have been two case reports of urticaria (Kulczycki, 1986) and two case reports of granulomatous panniculitis (Novick, 1985; McCauliffe and Poitras, 1991) thought to be related to aspartame. However, the pitfalls of single case reports, even those done under double-blind, placebo-controlled, crossover conditions, were highlighted by Geha (1992), who emphasized the importance of well-controlled clinical studies, rather than single case reports, to provide valid answers to medically important issues.

Animal Studies with Aspartame

Studies have shown that aspartame is not a direct mast cell or basophil secretagogue, either by *in vitro* methods or by skin testing *in vivo* (Szucs *et al.*, 1986). In addition, studies in rats have shown that aspartame has no effect on inflammation parameters such as carrageenan-induced paw edema, connective tissue formation, and adjuvant arthritis (Aspinall *et al.*, 1980). Nonetheless, in order to resolve the question of allergic-type reactions attributed to aspartame, controlled clinical trials were conducted.

Single-Site Study with Aspartame

Garriga *et al.* (1991) conducted a study with placebo-controlled oral challenges in patients who reported sensitivity to aspartame. Although extensive efforts were made to recruit subjects through local newspaper advertisements and interactions with local allergists and dermatologists, recruitment of suitable study subjects was extraordinarily difficult compared to the authors' experience in other studies of possible food sensitivity. As a result, only 61 initial subjects were identified after 32 months of enrollment. Upon screening, only 20 of these 61 patients had histories and symptoms to warrant evaluation by oral aspartame challenge.

Prior to oral challenge, each patient underwent skin testing to a panel of aeroallergens and some foods as well as both aspartame and diketopiperazine (DKP), a cyclic compound formed from aspartame under conditions of extremes in temperature and pH and long storage times in beverages. Of the 12 patients who enrolled, 9 had negative single-blinded challenges. Of the 3 patients with positive reactions to single-blind challenge, 1 developed hives, 1 reported "throat tightness," and 1 developed rhinitis. However, none of these reactions

were reproducible upon double-blind challenge. The authors concluded that it was difficult to find and study subjects thought to be sensitive to aspartame and that blinded oral challenge of patients with presumed aspartame sensitivity failed to result in reproducible results.

Multicenter Study with Aspartame

A multicenter, randomized, double-blinded, placebo-controlled, crossover clinical study conducted in clinical research units over a 5-day period was designed to evaluate if allergic-type symptoms could be attributable to aspartame (Geha *et al.*, 1993). Subjects were required to have a history of urticaria or angioedema within 12 h of consuming an aspartame-containing product during the previous 3 years or a history of chronic urticaria, which resolved without medication upon cessation of aspartame and recurred upon resumption of aspartame consumption.

Subjects were given, in random order, (1) capsules containing 25 or 300 mg aspartame, capsules containing 7.5 mg DKP, and 3.75 mg β -aspartame (conversion products of aspartame) with 13.75 mg microcrystalline cellulose and (2) identical placebo capsules containing 25 or 300 mg microcrystalline cellulose. The aspartame used in the study also contained approximately 0.32% DKP and 0.35% β -aspartame. Conversion products of aspartame, DKP and β -aspartame, were included in the study to determine if allergic-type reactions may be due to them rather than aspartame. Meals were standardized on both treatment days, and no aspartame-containing products were included. In addition, all food consumed on test days was recorded.

Subject recruitment efforts continued for over 4 years and included letters to over 4700 allergists in the United States and Canada, advertisements in two major allergy journals and 11 local allergy and dermatology societies, and contacts with 102 individuals who had filed complaints with The NutraSweet Company of urticaria and/or angioedema thought to be related to aspartame. However, despite these efforts, only 21 subjects, who were convinced that aspartame caused their allergic-type reactions, were enrolled into the study (Table 1). Following challenge, 17 of the 21 subjects had no allergic-type reactions. Four subjects experienced allergic-type reactions—two had reactions following ingestion of aspartame but not placebo, while the two others had reactions following ingestion of placebo but not aspartame. There was no statistically significant difference in the incidence of allergic-type reactions between aspartame and placebo challenges ($P = 1.00$) (Fig. 1). Of the positive reactions, only one individual experienced a severe reaction consisting of generalized urticaria, which persisted for 4 months and required treatment

TABLE 1

History of Allergic-Type Symptoms Attributed to Aspartame in Subjects Evaluated for Possible Allergic Reactions to Aspartame^a

Subject ^b	Urticaria	Angioedema	Chronic urticaria
A01	X	X	
A02	X	X	X
B01	X		
B02	X	X	
B03	X	X	
C01	X		
C03	X	X	
D01	X	X	X
D02	X	X	
E01	X	X	
E02	X		
E03	X		
E04	X	X	
E05	X		
E06	X		
F01	X		
F02		X	
F03	X		
F04	X	X	
F05	X		
F06	X		

^a Adapted from Geha *et al.* (1993).

^b Letters before subject numbers represent different academic centers in this multicenter study.

with H₁- and H₂-histamine blockers and oral corticosteroids. This occurred on the first treatment arm, which was placebo. Further, there was no statistically significant difference between aspartame and placebo treatments in the number of subjects with other adverse experiences.

The authors concluded that aspartame and its conversion products (DKP and β -aspartame) were no more likely to cause allergic reactions or other adverse experiences than placebo. Taken together, these findings sug-

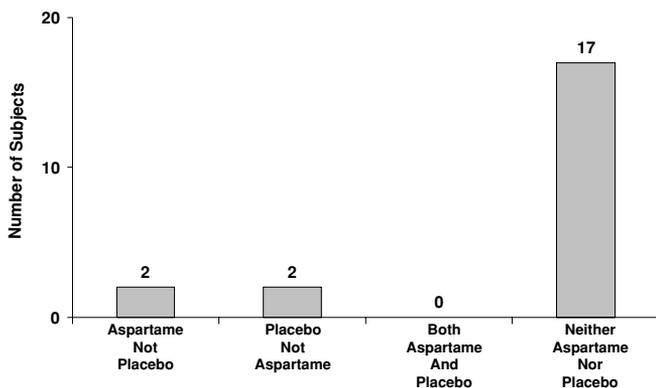


FIG. 1. Incidence of allergic-type reactions in 21 subjects after aspartame compared to placebo ($P = 1.00$). Source: Geha *et al.* (1993).

gest that allergic-type reactions to aspartame, if they exist, are very rare.

This study was criticized by Kulczycki (1995) because he felt that direct appeals to the public for cases was a more valid recruitment method than that used by Geha and co-workers (1993) of contacts with allergists and dermatologists. However, he neglected to take into account the suggestibility and bias introduced by direct appeals to the public; in addition, medical information from consumers in such circumstances is rarely as reliable as that from physicians who have evaluated the patient. Second, he criticized the fact that the Geha study was done in clinical research units over 5 days rather than in a short clinic visit as he had done in evaluating his case reports. On the contrary, the fact that Geha *et al.* did their study in the controlled conditions of clinical research units was a major strength of the study. In contrast, Kulczycki (1986) could not control what his subjects were exposed to once they left his clinic. Kulczycki (1995) also alleged that no effort was made to control for diet in the Geha *et al.* (1993) study, although the authors stated that meals were standardized on both treatments. On the contrary, there was no control of his subjects' diets once they left the clinic, which is especially troublesome in the case of the delayed reactions that he reported. Kulczycki also questioned whether a 3-week exclusion criteria for astemizole, which has a long half-life, was appropriate. Although it is not mentioned in the published paper, Geha *et al.* actually excluded patients taking astemizole for 8 weeks prior to the study while the national task force mentioned by Kulczycki had recommended 6 weeks. Thus, Kulczycki's criticisms are not appropriate or valid.

Pseudoallergy

Wedi and co-workers (1998) evaluated the relationship between *Helicobacter pylori* and chronic urticaria. Patients who were shown not to have a potential infectious cause of their urticaria were treated with a diet free of additives and known naturally occurring pseudoallergens (i.e., where the mechanism does not involve immunoglobulin E (IgE)). After symptoms improved with the diet, subjects were challenged under single-blinded, placebo-controlled conditions with increasing doses of food additives, including aspartame, in gelatin capsules. Only 3% of the people who benefited from the elimination diet developed urticaria to food additive challenges—two to potassium metabisulfite and one to sodium nitrite. None reacted to aspartame.

Occupational Exposure to Aspartame

In response to worker concern at a major U.S. food manufacturer regarding symptoms of possible occupational asthma among workers who were exposed to aspartame during manufacturing of food products,

the U.S. National Institute of Occupational Safety and Health (Burr and Barnard, 1992) conducted a series of site visits to assess work practices, assess employee exposure to airborne dusts, and conduct a medical evaluation. The medical evaluation included a self-administered employee questionnaire, spirometry, peak expiratory flow volume rates during waking hours both inside and outside the plant, skin prick tests with specific workplace antigens, measurements of serum IgE, and radioallergosorbent test (RAST) testing for possible aspartame sensitization.

The investigators found no difference between cases and controls in spirometry, peak expiratory flow volume in and out of the workplace, skin prick testing with specific antigens, serum IgE concentrations, or RAST. There were no positive skin tests with aspartame. In addition, there was no relationship between aspartame exposure and respiratory symptoms or changes in peak expiratory flow rates. There was also no dose-response relationship between respiratory symptoms recorded during peak expiratory flow rate testing and exposure to aspartame. The investigators concluded that they found no association between the symptoms of asthma and exposure to aspartame.

Conclusions

Early on, there were anecdotal reports and several single case reports suggesting that aspartame may be associated with allergic-type reactions. However, several clinical studies have been completed to address the issue raised by the case reports. When evaluated under controlled conditions, aspartame is no more likely to cause allergic reactions than placebo.

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Evaluation of Aspartame and Brain Tumors

Introduction

Before the approval of aspartame more than 20 years ago, Olney had suggested that aspartame may be associated with brain tumors based on his post hoc analysis of the results of long-term carcinogenicity studies in rats (FDA, 1981). After combining data from independent treatment groups in one study, he claimed there was a dose–response relationship between aspartame and brain tumors. Specifically, he combined data from different lower and higher dose groups to achieve an apparent dose response. He further speculated that the rate of spontaneous brain tumors in controls reported in another study was markedly higher than historical values, an incidence he placed at 0.1%. Olney's analysis and other issues were evaluated by scientists in the FDA Bureau of Foods as well as by a Public Board of Inquiry (PBOI) established by U.S. FDA. The PBOI was unable to reach a conclusion regarding aspartame and brain tumors. However, FDA scientists identified a number of issues with the PBOI's evaluation of Olney's assertions, including the historical incidence of brain tumors in controls being at least 20–30 times what Olney suggested, inappropriate combination of independent dose groups, incorrect statistical analysis, and errors in stated dates of animal deaths. Based on these considerations, when approving aspartame for human consumption, the FDA Commissioner and scientists within the Bureau of Foods concluded that aspartame does not cause brain tumors in rats (FDA, 1981).

Subsequently, after becoming aware of the flaws in Olney's analysis and learning of the results of a third carcinogenicity study in rats (Ishii, 1981) demonstrating no relationship between aspartame and brain tumors, the members of the PBOI agreed with the conclusions reached by FDA and endorsed FDA's decision to approve aspartame. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) (1980), the UK Ministry of Agriculture, Fisheries, and Food (1982), the Scientific Committee for Food in the EU (1985), the Canadian Health Protection Branch of Health and Welfare Canada (1981), and numerous other national regulatory agencies similarly concluded that aspartame is not a carcinogen and approved aspartame for use in foods.

In 1996, Olney again raised the issue of aspartame and carcinogenicity. On this occasion, Olney *et al.* (1996) claimed that the rate of brain tumors increased in the United States concurrent with aspartame use and postulated nitrosation of aspartame as a putative mechanism for a national increase in brain tumor rates. This purported association between aspartame and brain tumors was based on a flawed analysis of selected epidemiological data from the U.S. Surveillance, Epidemiology and End Results (SEER) tumor database maintained by the National Cancer Institute (NCI).

Olney *et al.* claimed they saw a biphasic pattern in age-adjusted brain tumor rates: one phase from 1975–1984 followed by a “striking jump” in 1985 and then a sustained (i.e., static) rate in the phase from 1985–1992. The authors hypothesized that this higher sustained rate for brain tumors was associated with the use of aspartame. However, the authors inexplicably omitted, without comment, the SEER data available for the years 1973 and 1974, which clearly demonstrate that the rising trend in rates of brain tumors predated aspartame approval. Earlier, Roberts (1991), another long-time critic of aspartame, had suggested a similar premise based on his own misinterpretations of the SEER data (Butchko, 1992). His contentions were also evaluated by FDA (FDA, 1992), which stressed that Roberts had misinterpreted the SEER data and that his assertions regarding the carcinogenicity studies in animals were false.

Considering its metabolism, it is not surprising that aspartame is not associated with brain tumors. As discussed in detail under the section “Metabolism of Aspartame,” aspartame is metabolized in the gastrointestinal tract to its three constituents—the amino acids, aspartate and phenylalanine, and methanol—which are then absorbed and utilized by the body through the same metabolic pathways as when these constituents are also derived from common foods.

Epidemiology of Brain Tumors in the United States

The arguments of Olney *et al.* (1996) require acceptance of two biologically implausible assumptions: first, that a certain factor (aspartame) could cause an increase in the incidence of brain cancer in less than 4 years (aspartame was not available in the United States until late 1981 when it was approved for dry uses and not widely used until after its approval in beverages by FDA in mid-1983) and second, that even more widespread exposure to this factor would cause no further increase in the incidence of that cancer in subsequent years. The trend of increased incidence of brain tumors started in the early to mid 1970s, well before aspartame was approved. Further, overall brain tumor rates from the SEER database had been reported to be decelerating (Levy and Hedeker, 1996). More recent SEER data (NCI, 1999) show that the overall percentage of change in incidence of brain tumors from 1975 to 1979, before aspartame was marketed, increased 1.6% whereas the percentage of change from 1992–1996, after aspartame was marketed, decreased –6.6%. Further, the estimated annual percentage of change was 0.6% for 1975–1979 vs –2.2% for 1992–1996, a statistically significant decrease. The age-adjusted brain tumor rates from the SEER registry (1973–1996) are included in Fig. 1.

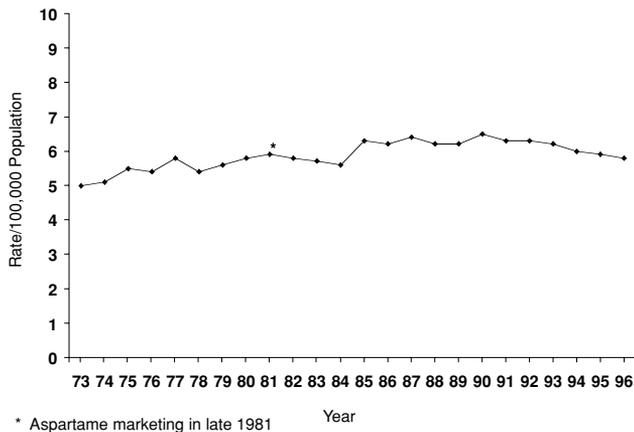


FIG. 1. Age-adjusted brain and other CNS tumor rates from 1973 to 1996 from the SEER registry (NCI, 1999). Increases in brain tumor rates started before aspartame marketing in 1981. *Source:* NCI, 1999.

In addition, increases in brain tumor rates have been primarily in the elderly, especially the very elderly (Muir *et al.*, 1994; Greig *et al.*, 1990; Werner *et al.*, 1995; Davis *et al.*, 1991). For example, Greig *et al.* (1990) reported a 500% increase in brain tumors in people 85 years and older. This has led to considerable debate regarding whether the reported increases in the elderly represent a “real” increase in brain tumor rates or whether other factors account for this observation (Muir *et al.*, 1994; Greig *et al.*, 1990; Werner *et al.*, 1995; Davis *et al.*, 1991; La Vecchia *et al.*, 1992; Boyle *et al.*, 1990; Marshall, 1990). This issue was critically reviewed by Modan *et al.* (1992), who concluded that the observed increases were not “real” but were related to enhanced detection resulting from “availability of more sophisticated noninvasive diagnostic technology; change in the attitude toward care of the elderly; and introduction of support programs such as Medicare that facilitate diagnostic procedures in the elderly.”

Legler *et al.* (1999) evaluated incidence data for brain and other central nervous system (CNS) tumors from the SEER results from 1975 to 1995 and concluded that, in contrast to earlier reports, there was a level or declining trend in brain tumor rates for all but the most elderly people. The fact that the elderly largely account for increases in overall brain tumor rates is clear from a recent SEER report (NCI, 1999). The percent change in brain tumor incidence for all ages from 1973 to 1996 was 15.8%; however, for ages under 65 years, the percent change was only 6.0%, whereas it was 46.5% for ages 65 and over during this time period (Fig. 2). As the most avid aspartame consumers are young and middle-aged adults (personal communication, confidential marketing data from MRCA Information Services, 1995), marked increases in brain tumor incidence in the elderly do not correlate with the demographics of aspartame use.

Smith *et al.* (1998) reported a 35% increase in brain tumors in children from 1973 to 1994 with a lower rate in brain tumors before 1984–1985 with a subsequent step increase. An important factor in the overall increase in malignant childhood brain tumors was an increase in low-grade gliomas, which may be accounted for by more ready detection of such tumors by MRI than by CT. The availability of MRI was very restricted prior to 1985. In addition, there was also a change in the classification system for childhood brain tumors during the mid-1980s whereby some tumors (e.g., low-grade gliomas) previously classified as benign were then classified as malignant and therefore subsequently included for the first time in the SEER database. There were also changes in neurosurgical practices (e.g., stereotactic biopsies) in the mid-1980s that may have led to increased diagnosis and reporting of brain tumors in children. These authors attributed this finding to changes in the mid-1980s in the detection and/or reporting of brain tumors in children. A subsequent evaluation of SEER data for childhood brain tumors by Linet *et al.* (1999) supported this conclusion.

Olney *et al.* (1996) also purported an increase in the malignancy of brain tumors after aspartame marketing. As with brain tumors in general, increases in highly malignant glioblastomas were primarily in the elderly in the SEER database (Levy and Hedeker, 1996) as has also been reported by Werner *et al.* (1995). Thus, increases in glioblastomas are largely accounted for by increases in the elderly and may be the result of the factors described by Modan *et al.* (1992). The increase in anaplastic astrocytomas referenced by Olney *et al.* is likely an artifact of recent shifts in classification terminology. Werner *et al.* (1995) cited wider acceptance of a three-tier classification system (astrocytoma, anaplastic astrocytoma, and glioblastoma) during the mid-1980s. Thus, some tumors classified as astrocytomas in the early 1980s would have been classified as anaplastic astrocytomas by the mid- to late 1980s.

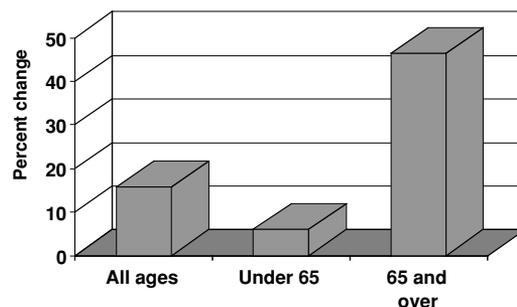


FIG. 2. Percentage of change in brain and other CNS tumor incidence for all ages, under age 65 years, and 65 years and older from 1973 to 1996 in the SEER registry (NCI, 1999). Much of the reported increase in brain tumor incidence in the United States has resulted from increases in the elderly population, which does not represent avid aspartame consumers. *Source:* NCI, 1999.

Further, if there were truly an increase in the malignancy of brain tumors, one would have expected that mortality from brain tumors would have risen. On the contrary, the percent change in mortality for all ages from 1975 to 1979 was an increase of 2.5% compared to a decrease of -2.1% from 1992 to 1996, and 5-year relative survival rates were significantly higher from 1989-1995 compared to 1974-1976 (NCI, 1999). Thus, changes in SEER data during the mid-1980s can be explained largely by changes in the classification system of brain tumors and the ability to diagnose tumors earlier due to the availability of advanced neuroimaging techniques and changes in neurosurgical practices, such as the availability of stereotactic biopsies. Declining mortality is not consistent with a true increase in brain tumors or increased malignancy of brain tumors. In Sweden, Hardell *et al.* (2000) included aspartame (as assessed by low-calorie drink consumption) in a case-control study of brain tumors and radiology work, medical X-ray investigations, and use of cellular phones. Only one of nine comparisons appeared to indicate an association between low-calorie drink intake and brain tumors. However, not all low-calorie drinks contain aspartame, and many are blends of sweeteners. Further, the authors stated that "the mean age of the cases and controls was 50 years, and consumption of low-calorie drinks is clearly a more common habit in young subjects." The authors concluded that with such a small number of subjects, these results "must be interpreted with caution."

In the United States, Gurney *et al.* (1997) evaluated aspartame consumption and the risk of childhood brain tumors in a case-control study. Case subjects were 19 years of age or older and were diagnosed with a primary brain tumor between 1984 and 1991. The authors concluded that children with brain tumors were no more likely to have consumed aspartame than were children in the control group. There was also no increased risk from maternal consumption of aspartame during pregnancy and no evidence of an association of specific types of brain tumors with aspartame. Gurney and co-workers concluded, "...it appears unlikely that any carcinogenic effect of aspartame ingestion could have accounted for the recent brain tumor trends as Olney *et al.* contend" (Gurney, 1997).

From an epidemiologist's standpoint, Davies *et al.* (1996) reviewed the paper by Olney *et al.* (1996) and concluded, "This report is most disturbing for those who practice epidemiology (this study was not done by an epidemiologist). The take-home message is completely speculative (as well as volatile), but to undo the damage that has already been done in the media is likely impossible." Ross (1998) concluded, "From an epidemiologic perspective, the conclusion of the report may well represent a classic example of 'ecologic fallacy' [reference 6 in the paper by Ross], because the Olney *et al.* study was a correlative analysis (i.e., ecologic analysis) that

demonstrated that two events occurred during roughly the same time period. There is no information available regarding whether the individuals who developed brain tumors consumed aspartame. For example, one might also invoke (a) cellular phone, home computer, and VCR usage; (b) depletion of the ozone layer; or (c) increased use of stereo headphones as potentially causative agents to argue trends in brain tumors and the changing environment. All such events could potentially be positively correlated with brain tumor incidence, and some or all of these possibilities may or may not have any biological plausibility to the observed associations." Recently, Weihrauch *et al.* (2001) reviewed potential carcinogenicity of high-intensity sweeteners. Regarding the allegations with aspartame, they concluded that, despite sensational and unscientific articles in the lay press and scientific literature, there is no well-founded proof of the carcinogenicity of aspartame.

Evaluation of Carcinogenicity in Animals

The three studies in rats and one study in mice done with aspartame to evaluate carcinogenicity were evaluated by regulatory bodies and expert scientists (JECFA, 1980; FDA, 1981; Health and Welfare Canada, 1981; MAFF, 1982; Ishii, 1981; Koestner, 1984, 1997; Cornell *et al.*, 1984); the conclusion is that aspartame is not a carcinogen, even after long-term administration of dosages hundreds to thousands of times greater than 90th percentile consumption by humans. The highest dose (8000 mg/kg body wt/day) used in these studies is the equivalent of an adult human consuming about 1000 liters of beverage sweetened with 100% aspartame daily over a lifetime. In addition, aspartame was not genotoxic when evaluated in the Ames test, the host-mediated assay in mice and rats, the dominant lethal mutation assay in rats, and the *in vivo* cytogenetics assay in rats (JECFA, 1980; Kotsonis and Hjelle, 1996).

As Olney had done in the late 1970s, Olney *et al.* in 1996 again argued that, in one study (E-33/34) when data from independent dose groups are combined, a dose-dependent higher rate of brain tumors can be observed in aspartame-fed rats compared to control rats. Further, they argued that the incidence of brain tumors in control rats was unreliable in another lifetime rat study, including *in utero* exposure to aspartame (E-70), because the observed incidence was higher than Olney's expectations. The underlying basis for such claims is the incorrect assertion by this long-time aspartame critic that the background incidence of brain tumors in Sprague-Dawley (SD) rats is 0.1%; the actual background incidence is at least 20-30 times higher (FDA, 1981; Koestner, 1980, 1984, 1997; Borzelleca *et al.*, 1985; Swenberg, 1986). In actuality, the overall incidence of brain tumors in the studies sponsored by G. D. Searle and Co. (Searle), the developer of aspartame, was

identical and well within background incidences in both of the contested studies (FDA, 1981).

Olney's assertions were evaluated in detail before the approval of aspartame in the United States. In 1980, the FDA convened a panel of academic experts, the PBOI (FDA, 1981), to evaluate several issues, including Olney's analysis of brain tumors in rats. In addition, prior to the PBOI, the FDA worked with the Universities Associated for Research and Education in Pathology (UAREP), a consortium of nine universities that was recognized for its expertise in the area of preclinical animal testing, to conduct a detailed audit to evaluate the validity of the Searle studies and to perform an independent reassessment of the incidence of brain tumors in these studies. UAREP and FDA concluded that the studies were valid for determining whether aspartame was a carcinogen (FDA, 1981; U.S. General Accounting Office, 1987).

Complicating PBOI's deliberations was the fact that the incidence of spontaneous brain tumors in Sprague-Dawley rats was not well characterized in the scientific literature at that time (FDA, 1981; Koestner, 1980, 1984, 1997). Technical differences made precise comparisons of available studies difficult. These differences included incomplete information on the age of the animals at the time of death and evaluation of only macroscopic tumors in some studies while others included histopathological examinations to identify microscopic tumors, histopathological evaluations done only on animals exhibiting neurological signs, examination of as few as one or two sections of brain, and differences in calculating background incidence. As Koestner concluded, "Since brain tumors are an age-related lesion in rats, and may only be present as microtumors at an age of two years (the time most studies are terminated), both age and thoroughness of histological examination significantly influence the detectable incidence of brain tumors" (Koestner, 1997).

In its evaluation, the PBOI cited four studies with brain tumor incidence rates ranging from 0.09, 0.6, 0.7, and 3.2% but elected to use a rate of 0.7% as the historical basis for spontaneous brain tumors in Sprague-Dawley rats (FDA, 1981). Thus, based on this historical brain tumor rate, the members of the PBOI were unable to rule out a possible carcinogenic effect of aspartame. However, additional studies available in the FDA administrative record were not considered by the PBOI, and other studies became available after PBOI's deliberations. These studies reported spontaneous incidences of brain tumors of up to 5% in control rats.

FDA concluded that variability in the spontaneous brain tumor rates would be expected among bioassays done at different times, using different procedures, conducted at different places and by different people. For example, the results of a series of studies done by the NCI using animals from the same source as those in the Searle studies and housed, at least in part, in the same

laboratory as the Searle studies, reported a spontaneous brain tumor rate of 2.2%. Further, more detailed histopathologic examinations (eight brain sections per animal) were done in the Searle studies than was typically done (two to three brain sections per animal) at that time or even today. The greater number of brain sections evaluated increase the chance of finding microscopic tumors, and thus, the background incidence of tumors would be expected to be greater than in similar studies where smaller numbers of brain sections are examined. Microscopic tumors actually comprised most of the brain tumors in the Searle studies (Koestner, 1980, 1984, 1997), leading Koestner to conclude, "...most of the tumors would have been missed with a less qualified examination" (Koestner, 1997). Based on these factors, FDA considered the background incidence used by the PBOI as unduly low and concluded that the technically adequate historical control data were consistent with the rates observed in the Searle studies. Subsequently, additional information available in the scientific literature again confirmed that spontaneous brain tumor rates in Sprague-Dawley rats are quite variable and range from 0 to 11% (Table 1) (Svenberg, 1986; Koestner, 1997; Borzelleca *et al.*, 1985). Today, the rate of spontaneous brain tumors in Sprague-Dawley rats at 2 years of age is generally accepted to be approximately 3% (Koestner, 1997). Regardless of the rates of brain tumors in historical controls, FDA considered that the most appropriate comparison for treatment groups is concurrent controls within the same study. The rate of brain tumors in the rat study with *in utero* exposure to aspartame (E-70) is consistent with both historical and concurrent controls (FDA, 1981).

Another critical error in Olney's analysis was the inappropriate combination of data from independent dose groups in an apparent attempt to show a dose-response relationship between aspartame and brain tumors in the other rat study (E-33/34). The PBOI seriously considered Olney's post hoc analysis of this study combining data from independent dose groups and concluded that there did appear to be a dose-related increase in brain tumors in Olney's analysis. However, FDA used a standard and accepted analysis of carcinogenicity studies including appropriate statistical methods and determined there was clearly no dose-dependent increase in brain tumors in the two Searle studies (Tables 2 and 3). This evaluation was done comparing the concurrent controls versus treatment groups separately for each sex. Since the medulloblastoma was considered of embryonal origin and not related to treatment, it was not included in the evaluation.

FDA further enumerated several key errors made by the PBOI in assessing the data from the aspartame carcinogenicity studies (FDA, 1981; U.S. General Accounting Office, 1987). The Canadian Health Protection Branch concurred with FDA regarding these errors (Health and Welfare Canada, 1981). These errors

TABLE 1
Variability in Brain Glioma Incidence in Control Male Sprague–Dawley Rats
(Three Brain Sections Unless Specified)^a

Study	Color	Laboratory	Control 1 (%)	Control 2 (%)	Control 3 (%)
1–5	Different colors	IRDC	0/292 (0)	2/287 (0.7) ^b	2/117 (1.7)
6	Red No. 33	IRDC	3/57 (5.3)	0/59 (0)	2/58 (3.4)
7	Green No. 3	Biodynamics	0/52 (0)	5/55 (9.1) ^c	—
8	Blue No. 2	Biodynamics	0/59 (0) ^d	2/59 (3.4)	—
9–13	Different colors	Biodynamics	2/290 (0.7)	2/289 (0.7)	4/231 (1.7)
14	Red No. 9	Litton	4/58 (6.9)	6/60 (10)	2/57 (3.5)
15	Red No. 27	Litton	2/54 (3.7)	0/55 (0)	—
16	Red No. 36	Litton	2/57 (3.5)	1/59 (1.7)	0/53 (0)
17	Red No. 30	Hazleton	3/59 (5.1)	1/55 (1.8)	—

^a Adapted from Swenberg (1986).

^b One of the rats died on day 350 with a glioma.

^c Additional sections resulted in 6/55 (10.9%).

^d Additional sections resulted in 2/59 (3.4%).

TABLE 2
Brain Tumors in the 2-Year Carcinogenicity Study with Aspartame
in Sprague–Dawley Rats (E-33/34)^{a,b}

Dose (mg/kg body wt/day)	Number of males	Number and type of brain tumors in males	Number of females	Number and type of brain tumors in females
0	59	1 (astrocytoma)	59	0
1000	36	1 (astrocytoma)	40	1 (astrocytoma)
2000	40	1 (astrocytoma)	40	1 (astrocytoma)
4000	40	1 (astrocytoma)	40	0
		1 (oligodendroglioma)		1 (astrocytoma)
		1 (astrocytoma)		
		1 (astrocytoma)		
6000–8000	39	1 (astrocytoma)	38	1 (medulloblastoma) ^c
		0		1 (astrocytoma)

^a From FDA, 1981, E-33/34, E-87, and E-102 (UAREP).

^b E-33/34 was originally evaluated with two sections per brain but was re-assessed with eight sections per brain (E-87) and then by UAREP (E-102).

^c Animal died during week 13 of the study (about 16 weeks of age); this medulloblastoma was considered to be embryonal in origin and not related to treatment.

TABLE 3
Brain Tumors in the 2-Year Carcinogenicity Study Including *in utero* Exposure
with Aspartame in Sprague–Dawley Rats (E-70)^{a,b}

Dose (mg/kg body wt/day)	Number of males	Number and type of brain tumors in males	Number of females	Number and type of brain tumors in females
0	58	1 (astrocytoma)	57	1 (astrocytoma)
		1 (astrocytoma)		
		1 (astrocytoma)		
2000	36	1 (ependymoma)	39	1 (astrocytoma)
		1 (astrocytoma)		
4000	40	1 (astrocytoma)	40	1 (meningioma)

^a From FDA, 1981, E-70, E-87, and E-102 (UAREP).

^b Eight sections per brain were evaluated.

included suggesting a dose–response relationship based on an inappropriate combination of dose groups, misdiagnosing a primary brain tumor as metastatic when no other tissues or organs in the animal had carcinoma, misunderstanding the expert testimony related to one medulloblastoma and ascribing significance to this tumor, making errors in death dates of some animals, speculating on the size of tumors and their role in the cause of death of animals without adequately reviewing the study data, and misunderstanding scientific publications relating to the spontaneous incidence of brain tumors in rats. For example, in E-33/34, the animal with a medulloblastoma died during Week 13 of the study or about 16 weeks of age. Medulloblastomas are of embryonal origin and very rare in rats. Based on the size of the tumor, the tumor was considered to have started in the embryonal stage of development. Thus, the tumor was eliminated from the analysis by FDA as definitely unrelated to aspartame. Supporting this conclusion was the fact that no such tumors were seen in E-70, where animals were transplacentally exposed to aspartame *in utero* (FDA, 1981; Koestner, 1980, 1984, 1997).

At the time of approval, the FDA Commissioner (FDA, 1981) concluded that the Searle studies (E-33/34 and E-70) were negative and stated, “. . .the incidence rates reported in the Searle studies fall within reasonably expected bounds of spontaneous incidence for the type of rat and study size used, and that the primary evaluation of these studies should be between the treated animals and their concurrent controls.” In addition, FDA concluded, “. . .the data in E-33/34 do not suggest, in terms of biological significance, a dose–response relationship or early tumor onset.”

Thus, the results of both rat studies supported the conclusion that aspartame is not carcinogenic. Further, an additional study done in mice (E-75) demonstrated that aspartame is not carcinogenic (FDA, 1981), and subsequently, a third, 2-year study in Wistar rats further confirmed that aspartame is not carcinogenic (Table 4) (Ishii, 1981).

Neurocarcinogens are known to exhibit a number of characteristic features (Koestner, 1980, 1984, 1997;

FDA, 1981). These biological criteria include the ability to increase tumor incidence beyond expected control levels, the demonstration of a dose–effect relationship, the occurrence of tumors at a younger age, a greater effect on embryonal and fetal cells than on adult neuroectodermal cells, and a shift to more anaplastic tumor types. After thorough analysis of aspartame studies, aspartame does not meet any of these criteria (Koestner, 1980, 1984, 1997; FDA, 1981).

Further, there is no evidence that aspartame increases the malignancy of brain tumors. The tumors in the aspartame studies were primarily mature, differentiated, and of glial origin. Specifically, none of the tumors were anaplastic; there was no resemblance to glioblastoma multiforme in humans, as was implied by Olney *et al.* (Koestner, 1997).

The potential mutagenicity and carcinogenicity of aspartame’s conversion product, cycloaspartylphenylalanine diketopiperazine (DKP), was also extensively evaluated (see “Metabolism of Aspartame” section for additional information on DKP). DKP was not genotoxic when evaluated in the Ames test, the host-mediated assay in mice and rats, the dominant lethal mutation assay in rats, and an *in vivo* cytogenetics assay in rats (JECFA, 1980; Kotsonis and Hjelle, 1996). Two-year carcinogenicity studies in rats and mice were done with doses of DKP up to 3000 mg/kg body wt/day, which is over 5000 times estimated 90th percentile daily DKP intake from aspartame in the United States (Kotsonis and Hjelle, 1996). In the 115-week carcinogenicity study in Sprague–Dawley rats (E-77/78), DKP was administered in the diet to provide dosages of 0, 750, 1500, and 3000 mg/kg body wt/day. Seven sections per brain were evaluated microscopically, and the incidence of brain tumors in males were 2 astrocytomas/56 in controls, 1 oligodendroglioma/33 at 750 mg/kg body wt/day, and 0/32 and 0/31 at 1500 and 3000 mg/kg body wt/day, respectively. In females, the incidence was 0/67, 0/35, and 0/34 at 0, 750, and 1500 mg/kg body wt/day, respectively, and 1 astrocytoma and 1 oligodendroglioma/33 animals at 3000 mg/kg body wt/day. There was no effect of DKP on brain tumors or any other evidence of carcinogenicity

TABLE 4
Brain Tumors in the 2-Year Carcinogenicity Study with Aspartame and Diketopiperazine (DKP) in Wistar Rats^{a,b}

Dose (mg/kg body wt/day)	Number of males	Number and type of brain tumors in males	Number of females	Number and type of brain tumors in females
Control	59	0	60	1 (atypical astrocytoma)
1000	59	1 (oligodendroglioma)	60	0
2000	60	0	60	1 (astrocytoma)
				1 (ependymoma)
4000	60	1 (astrocytoma)	60	0
3000 + 1000 DKP	60	0	60	1 (oligodendroglioma)

^a Adapted from Ishii (1981).

^b Six sections per brain were evaluated.

(FDA, 1981; JECFA, 1980). There were also no carcinogenic effects in the mouse study (E-76) (Kotsonis and Hjelle, 1996; FDA, 1981). In addition, another study (Ishii, 1981) evaluated aspartame with DKP (3:1) in the diet of Wistar rats for 2 years. This study also demonstrated no evidence of carcinogenicity of DKP.

In granting the approval of aspartame for its intended use in 1981, the FDA Commissioner stated, "For all the reasons stated above, I conclude that the available data, taken as a whole, establish that there is a reasonable certainty that aspartame and DKP do not cause brain tumors in laboratory rats. This conclusion is based on studies E-33/34, E-70, and E-77/78, all of which were considered at the hearing [PBOI]. Additional support for this conclusion is found in the Japanese study [Ishii], submitted by Searle after the Board issued its decision. Accordingly, under the act's general safety clause, I find that the available data establish the safety of aspartame, in terms of brain tumors, for its proposed use."

The Health Protection Branch in Canada (Health and Welfare Canada, 1981) also evaluated Olney's assertions and came to the same conclusion as FDA, outlining a number of errors in the analysis and PBOI's assessment. The Canadian government concluded "that there is no association between aspartame ingestion and the incidence of brain neoplasms in the rat."

After approval of aspartame by FDA, the members of the PBOI became aware of the additional data and FDA's analysis. In a letter dated August 6, 1981 (Nauta, 1981), the Chairman of the PBOI wrote to the FDA Commissioner stating, "... had we known earlier about the reassuring outcome of the recent Japanese [Ishii] oncogenicity studies, our recommendation would doubtless have been for unqualified approval... we wish to express our endorsement of your final decision in this matter."

Purported Mechanism

A series of experiments done prior to the approval of aspartame showed no direct evidence of aspartame nitrosation under various harsh chemical conditions (FDA, 1983, 1984). In addition, no *N*-nitroso compounds formed *in vitro* or *in vivo* in several special studies done to evaluate the possible nitrosation of DKP (FDA, 1983, 1984; JECFA, 1980). Nonetheless, Olney *et al.* (1996) claimed that nitrosation studies by Shephard and co-workers (1993) provide a mechanistic explanation for how aspartame could act as a carcinogen (for additional perspective, see Shephard and Lutz, 1989; Shephard *et al.*, 1991). Olney *et al.* (1996) cited a report by Shephard *et al.* (1993) in which extremely high nitrite concentrations reacted with a variety of amino acids or peptides, including aspartame, apparently generated compounds with mutagenic properties when incubated with bacteria alone. However, when these compounds were incubated with liver enzymes to mimic

in vivo conditions, the mutagenicity of these compounds was markedly reduced (Shephard *et al.*, 1993). Further, the chemical conditions of the reactions required to produce these products are not unique to aspartame and are not relevant to aspartame consumption. The nitrite concentrations used were at least 10,000 times greater than actual human gastric levels (Challis *et al.*, 1982). As a result, the rate of the reaction reported by Shephard *et al.* (1993) was 100 million times greater than that possible in the human stomach (the reaction rate is dependent on the square of the nitrite concentration) (Flamm, 1997). In fact, Meier *et al.* (1990), from the same laboratory, concluded that the formation of nitrosoaspartame under standard conditions of use of aspartame as a sweetener would be "negligible in comparison with the dietary intake of preformed nitroso compounds." Furthermore, any possible reaction product(s) of aspartame would be the same as those formed by common dietary proteins, peptides and/or amino acids, which are present in the diet at far higher concentrations than aspartame. As a practical matter, the studies show that nitrosation of aspartame in the human stomach is negligible and will have no effect on the total body burden of nitrosamines whether they be preformed or formed in the stomach (Flamm, 1997).

Recent Evaluations by Regulatory and Government Agencies

The allegations by Olney *et al.* (1996) that aspartame may be associated with brain tumors have been evaluated by scientists at regulatory and government agencies in several countries. The U.S. National Cancer Institute (1997) concluded, "a recent analysis of the NCI statistics on cancer incidence in the United States does not support an association between the use of aspartame and an increased incidence of brain tumors." The U.S. FDA concluded that the analysis "does not support an association between the use of aspartame and increased incidence of brain tumors" (FDA, 1996). The Committee on Carcinogenicity at the Department of Health in the United Kingdom (Department of Health, 1998; Food Standards Agency, 1999; Caseley and Dixon, 2001) arrived at the same conclusion as FDA stating, "The Committee concluded that the data published by Olney *et al.* did not raise any concerns with regard to the use of aspartame in the United Kingdom" (Department of Health, 1998). The Scientific Committee for Food of the European Union concluded, "... the data do not support the proposed biphasic increase in the incidence of brain tumors in the USA during the 1980's" (European Commission, 1997). From their analysis, the Australia/New Zealand Food Authority (ANZFA) concluded, "From the extensive scientific data available at this stage, the evidence does not support that aspartame is carcinogenic in either animals or humans. There appears to be no foundation to recent USA

reports of increased brain tumors in humans" (ANZFA, 1997).

Conclusions

In the late 1970s, Olney raised questions regarding the results of the carcinogenicity studies with aspartame, suggesting that the data showed an increase in brain tumors in rats. However, upon review of the data, scientists in regulatory agencies around the world disagreed with Olney's contentions and approved aspartame. In 1996, Olney *et al.* again raised this issue, this time asserting that an apparent increased incidence of human brain tumors in the United States may be associated with aspartame. Specifically, Olney *et al.* claimed that the rate of brain tumors increased in the United States concurrent with aspartame approval, that brain tumors increased in rats fed aspartame, and that nitrosation of aspartame is a putative mechanism whereby aspartame may cause brain tumors. Olney and colleagues claimed a "surge in brain tumors in the mid 1980s" based on their analysis of selected data from the U.S. Surveillance, Epidemiology and End Results (SEER) tumor data base from the U.S. National Cancer Institute (NCI).

The NCI, which maintains the SEER database, concluded that Olney's claims were not valid, as did scientific and regulatory bodies in the European Union, the United States, and other countries. The scientific consensus is that there is no association between aspartame consumption and an increase in human brain tumor rates, aspartame is not an animal carcinogen, and the speculated biochemical mechanism for carcinogenicity is not relevant to aspartame consumption. Thus, the evidence is clear that aspartame is not associated with brain tumors.

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Use of Aspartame by Potentially “Sensitive” Populations

Heterozygotes for Phenylketonuria

Individuals with the rare genetic disease phenylketonuria (PKU) (OMIM 261600) have a severely compromised ability to metabolize dietary phenylalanine and, consequently, have extreme elevations in plasma phenylalanine concentrations. If this disorder is not detected and dietary restriction of phenylalanine is not begun early in life, a variety of severe developmental and neurological sequelae will occur. (For a comprehensive review of PKU, see Scriver and Kaufman, 2001). Individuals heterozygous for PKU (PKUH) are carriers for this disease and have a somewhat diminished ability to metabolize dietary phenylalanine, which results in marginally higher than normal plasma phenylalanine concentrations. However, such individuals do not need restriction of dietary phenylalanine and have none of the neurologic sequelae associated with untreated PKU. Thus, they go undetected until two PKU heterozygous parents reproduce a homozygous PKU offspring. Because of the phenylalanine component of aspartame, concerns were raised regarding whether there was potential vulnerability of PKUH to the alleged effects of aspartame on brain function.

Tolerance of aspartame in PKUH. Before approval of aspartame, short-term and long-term tolerance of aspartame in PKUH were evaluated in a randomized, double-blinded manner (Koch *et al.*, 1976). During the 6-week phase, subjects received increasing (increased weekly) doses of aspartame (600–8100 mg/day); in the subsequent 21-week phase, subjects received 1800 mg aspartame/day. During the studies, subjects were evaluated via physical examinations, including ophthalmologic examinations, plasma phenylalanine and tyrosine concentrations, hematology evaluations, biochemical evaluations including tests of liver function and lipids, thyroid function, glucose and insulin concentrations, and urine chromatographies. No medical or biochemical changes were found after aspartame administration compared to placebo during the 27 weeks of the study; in addition, plasma phenylalanine and tyrosine concentrations remained within the normal range. The authors concluded that aspartame was well tolerated by PKUH.

Pharmacokinetics of phenylalanine from aspartame in PKUH. The metabolic effects of bolus doses of aspartame (4, 10, 34, and 100 mg/kg body wt) (Stegink *et al.*, 1979, 1980, 1987), repeated doses of aspartame (Stegink *et al.*, 1989, 1990), and aspartame given with a protein meal (Stegink *et al.*, 1991; Curtius *et al.*, 1994; Stegink and Filer, 1996) have been extensively evaluated in PKUH. (see “Metabolism of Aspartame” section for further details.) For perspective, the 100 mg/kg

body wt dose of aspartame is equivalent to a 70 kg adult consuming approximately 14 liters of beverage sweetened with aspartame in a single sitting.

From the results of the bolus dosing studies, PKU heterozygotes clear phenylalanine at about half the rate of normal individuals; however, even high intakes of phenylalanine from aspartame are adequately metabolized by PKUH (Stegink *et al.*, 1979, 1980, 1987). For example, a bolus dose of 34 mg/kg body wt aspartame (Stegink *et al.*, 1979) resulted in mean peak plasma phenylalanine concentrations within the normal postprandial range. Even after 100 mg/kg body wt aspartame (Stegink *et al.*, 1980), high mean plasma phenylalanine concentrations in PKUH ($41.7 \pm 2.33 \mu\text{mol/dl}$) were well below those associated with PKU.

In multiple dose studies in PKUH (Stegink *et al.*, 1989, 1990), mean peak plasma phenylalanine concentrations increased significantly over baseline after aspartame but approximated the normal postprandial range. The results of these studies demonstrate that a PKUH could ingest large amounts of aspartame at hourly intervals around the clock without increasing plasma phenylalanine concentrations appreciably above the postprandial range. Finally, as in healthy subjects, dietary protein modulates the rise in plasma phenylalanine concentrations after aspartame ingestion in PKUH (Stegink *et al.*, 1991; Curtius *et al.*, 1994; Stegink and Filer, 1996) compared to concentrations that would be expected from the dose of aspartame administered alone.

Brain function in PKUH. In two studies from one laboratory (Elsas and Trotter, 1988; Epstein *et al.*, 1989), a small number of PKUH were exposed to 100 mg/kg body wt phenylalanine daily in addition to the normal dietary intake of 60 mg/kg body wt. There was a suggestion that, when exposed to these large doses of phenylalanine, subjects had a decrease in mean power frequency on their EEGs. However, it is not clear that this observation, even if causally related and reproducible, has any clinical significance.

Thus, a randomized, double-blind, placebo-controlled, crossover study (Trefz *et al.*, 1994) was done in a larger number of PKUH subjects ($N = 48$) for 12 weeks duration each with both aspartame and placebo treatments. During the aspartame treatment arm, subjects were given either 15 or 45 mg/kg body wt/day of aspartame. These doses of aspartame are 5–15 times 90th percentile intake of aspartame (Butchko and Kotsonis, 1991). Subjects were tested with the Amsterdam Neuropsychological Tasks battery (de Sonneville, 1999), including tests for visual information processing and executive function of varying complexity. This computerized test battery has demonstrated changes in cognitive performance to be associated with

changes of plasma phenylalanine concentrations in individuals with PKU (Schmidt *et al.*, 1994), even in a very well-controlled population (Huijbregts *et al.*, 2002). In addition, to evaluate the earlier suggestions that aspartame may affect the mean power frequency of EEGs, conventional analysis of EEGs as well as analysis of the spectra by fast Fourier transformation, a more sophisticated analysis than used in the earlier studies, were done. There were no statistically significant differences found on routine EEG analysis, EEG spectral analysis, or neuropsychological tests between either of the aspartame treatments and placebo. In addition, there was no difference in adverse experiences after aspartame compared to placebo.

Depressed Individuals

Walton *et al.* (1993) reported an increase in the occurrence of adverse experiences after aspartame vs placebo in a population, which they felt may be potentially vulnerable to aspartame consumption, i.e., people with monopolar depression. The study design was a double-blind, placebo-controlled, crossover design where doses of aspartame of 30 mg/kg body wt/day or placebo were given for 7 consecutive days on each treatment. The authors aborted the study after two serious ophthalmologic complications were observed. One of these occurred in the placebo arm and one in the aspartame arm. The authors reported significant differences between aspartame and placebo in reported adverse experiences. However, the reliability of this study has been questioned (Schomer *et al.*, 1996; Butchko, 1994). Since the study was terminated after only 11 of the proposed 40 subjects completed their participation, too few subjects were evaluated to obtain any statistically valid conclusions. Further, in order to attain sufficient numbers and a statistically significant result with adverse experiences, complaints were consolidated across a range of unrelated categories, and subjects who had been dropped for adverse experiences were reentered for the analyses of complaints. The methodological errors present in the report and the apparent post hoc analysis are so serious that it is impossible to draw any conclusions from this study.

Movement and Motor Disorders

In patients with Parkinson's disease with fluctuating symptoms, there is an inverse relationship between plasma LNAA concentrations and motor performance as LNAAs compete with levodopa for entry into the brain. Thus, some Parkinson's patients achieve a better therapeutic response to levodopa if they also are treated with a protein-restricted diet. Because aspartame provides one of the LNAAs, phenylalanine, it was suggested that aspartame may adversely affect motor performance in such patients. This was evaluated in a double-blind, placebo-controlled, crossover study with

bolus doses of 600 and 1200 mg aspartame and placebo in levodopa-treated Parkinson's patients with protein-sensitive motor fluctuations (Karstaedt and Pincus, 1993). Although there were statistically significant increases in plasma phenylalanine concentrations after the 1200-mg dose of aspartame, there were no statistically significant effects of aspartame compared with placebo on motor performance.

In one case study where the lead investigator was also the single subject, he reported shaking of his arms and legs and various other vague symptoms continuing for several weeks after a 300-mg aspartame dosage (Gerrard *et al.*, 1994). However, such symptoms have not been reported elsewhere or in controlled studies.

Individuals with Dizziness

Review of the consumer anecdotal reports of symptoms possibly associated with aspartame by the CDC (1984) revealed that dizziness was a frequently reported symptom. Gulya and co-workers (1992) asked 53 patients who visited their clinic complaining of dizziness to complete a questionnaire regarding dietary habits thought to influence dizziness. Only seven patients, six of whom consumed aspartame, completed the questionnaire. Subjects were then asked to cease aspartame intake for 1 month and keep a log of their episodes of dizziness. Only one subject appeared to respond to cessation of aspartame intake; however, he did not participate in the crossover phase where aspartame would have been allowed. In other clinical studies with aspartame, including long-term studies, there was no association of aspartame with dizziness (Tschanz *et al.*, 1996).

Individuals with Renal Disease

Individuals with renal disease are an especially important population as they can have altered amino acid profiles and, as many of them are diabetics, would also be likely to consume aspartame, which provides two amino acids, aspartic acid and phenylalanine. Gupta *et al.* (1989) studied 23 diabetic patients with renal failure, who were on maintenance hemodialysis, to evaluate the effect of aspartame (10 mg/kg body wt), placebo, and the postprandial condition on plasma amino acid profiles in a randomized, double-blind, crossover study. As expected, plasma phenylalanine concentrations at 1 and 2 h after aspartame were significantly higher than after placebo but were within the normal postprandial range in these subjects. Plasma concentrations of tyrosine were also higher after aspartame vs placebo but were below the normal postprandial range in these subjects. There were no adverse experiences associated with aspartame. These authors concluded that aspartame is safe for diabetic subjects with chronic renal failure.

Individuals with Liver Disease

Phenylalanine has been believed to cause or affect hepatic encephalopathy in individuals with liver disease. Consequently, there was concern that such individuals may be affected by aspartame because of its amino acid constituents and also that such individuals may not properly metabolize its methanol component. The clinical and biochemical effects of aspartame (15 mg/kg body wt), skim (no-fat) milk (10 oz. containing phenylalanine in an equimolar amount with aspartame dose), and placebo were evaluated in individuals with chronic, alcoholic liver disease in a randomized, crossover study (Hertelendy *et al.*, 1993). As expected, aspartame resulted in a significantly greater increase in plasma phenylalanine concentrations compared to skim (no-fat) milk and placebo (C_{\max} , 14.55 ± 7.38 , 10.95 ± 4.95 , 8.84 ± 4.55 $\mu\text{mol/dl}$, respectively). There were no changes from baseline in plasma or blood aspartic acid, methanol, formate, or ammonia concentrations after any of the three treatments. Intensity of encephalopathy was evaluated by the portal systemic index (PSE), comprised of assessments of mental status, asterixis, Reitan-A trail test, blood ammonia concentrations, and EEG activity. There was significant worsening in PSE, primarily in mental status assessment only after the skim (no-fat) milk treatment. The authors concluded that aspartame may be used safely by individuals with chronic, stable liver disease.

Conclusion

Several subpopulations of individuals, including PKUH, individuals with depression, individuals with dizziness, individuals with Parkinson's disease, individuals with renal disease, and individuals with liver disease, who may be potentially sensitive to aspartame because of its amino acid content, have been studied. Extensive metabolism studies in PKUH using various paradigms have demonstrated that aspartame, even in amounts well above average 90th percentile consumption levels, results in plasma phenylalanine concentrations that are considered safe. In addition, tolerance studies and detailed evaluation of brain function in this population have shown no adverse effects of aspartame. A prematurely terminated study in depressed patients appeared to indicate that aspartame was associated with more adverse experiences than placebo; however, methodological flaws and the statistical analysis used raised serious questions regarding the validity of the study. In a study in patients with dizziness, only a few subjects completed the questionnaire, and only in one subject, who did not complete both treatment arms of the study, was there a suggestion that aspartame may have been associated with his dizziness. No effects of aspartame were seen in individuals with Parkinson's disease, whose symptoms and responsiveness to levodopa are sensitive to fluctuations in plasma amino acid concentrations. Finally, aspartame was demonstrated

to be safe for individuals with renal disease and individuals with liver disease.

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Endocrine Evaluations with Aspartame

Introduction

Because individuals with diabetes mellitus would likely be enthusiastic consumers of aspartame, there have been a number of studies done to evaluate aspartame's safety in this subpopulation. Studies included both acute and long-term dosing paradigms, with special emphasis on potential effects on plasma glucose and insulin concentrations. In addition, the effect of aspartame on plasma glucose and insulin concentrations has been evaluated in nondiabetic subjects in some studies. It had been speculated (Rogers *et al.*, 1988) that sweetness, even without the calories of sugar, may induce a cephalic-phase insulin release impacting blood glucose concentrations which may possibly be associated with increased hunger. Thus, several studies concentrated on this issue. Because certain amino acids may affect the release of other hormones (e.g., prolactin, cortisol, or growth hormone), studies have been undertaken to determine if large doses of aspartame affect hormone release.

Acute Dosing Studies with Aspartame in Normal and Diabetic Subjects

Several studies have been completed to evaluate the effect of acute doses of aspartame in diabetic subjects. Shigeta *et al.* (1985) evaluated the effect of 225 mg aspartame or 75 g of oral glucose on plasma glucose and insulin concentrations in subjects with Type 2 diabetes mellitus. Aspartame had no effect on plasma glucose or serum insulin concentrations. When aspartame was substituted for sucrose for 3 days in the diets of these subjects, there was no effect on fasting plasma glucose concentrations.

Okuno *et al.* (1986) studied the effect of 100 g of glucose or 500 mg of aspartame on blood glucose and insulin concentrations in normal subjects and Type 2 diabetics. Diabetic subjects were separated into three groups according to the severity of their fasting hyperglycemia. After the oral glucose load, there were the expected increases in blood glucose and serum insulin concentrations in proportion to the severity of fasting hyperglycemia. However, in all groups the ingestion of 500 mg of aspartame was associated with a decline in glycemia which was likely due to fasting, which occurred without observable changes in serum insulin or plasma glucagon concentrations.

Horwitz *et al.* (1988) studied the effects of single doses of aspartame (400 mg) and saccharin (135 mg) in beverage vs an unsweetened beverage on hormonal and glycemic responses in normal subjects and subjects with Type 2 diabetes. These doses of aspartame and saccharin are approximately equal to the amount of each sweetener in 1 liter of beverage. There were no signifi-

cant effects of either aspartame or saccharin on plasma glucose concentrations compared to unsweetened beverage at any time point in either the normal or diabetic subjects. Specifically, there were no differences between the three treatments in glucose area under the curve (AUC), C_{max} , or t_{max} in either normal or diabetic subjects (Tables 1 and 2). In both normal and diabetic subjects, however, there was a slight decline in glucose concentrations during the 180-min study period, which was similar among the treatment groups. In normal subjects, the AUC for insulin was greater after aspartame than after saccharin and unsweetened beverage ingestion. However, there were no differences among treatments in peak insulin concentrations or the time to reach the peak. The observed differences were small and likely related to a decrease from baseline insulin concentrations after both the unsweetened and the saccharin beverage and no change from baseline after aspartame beverage. In diabetic subjects, there were no differences in insulin concentrations as far as peak concentrations, time to peak, and AUC. Glucagon concentrations were variable, but there was no change in the overall magnitude of secretion (Horwitz *et al.*, 1988).

The effect of aspartame on plasma glucose and insulin concentrations was also evaluated in several metabolism studies using doses of aspartame ranging from approximately 4 to 40 mg/kg body wt (Wolf-Novak *et al.*, 1990; Møller, 1991; Stegink *et al.*, 1990). Aspartame had no effect on plasma insulin concentrations. Wolf-Novak *et al.* (1990) and Stegink *et al.* (1990) also reported no effect of aspartame on plasma glucose concentrations. Although Møller (1991) reported a significant effect of aspartame on plasma glucose concentration, such an effect is not consistent with the results of the studies by Wolf-Novak *et al.* (1990) and Stegink *et al.* (1990). A difference in baseline glucose concentrations between aspartame and the "water condition" likely affected the results in the study by Møller (1991).

Overduin and Jansen (1997) used aspartame as the control in a study to evaluate whether "conditioned" insulin and glucose responses were related to binge eating in healthy females. They used a sixfold pairing of a

TABLE 1
Plasma Glucose Pharmacokinetics (Mean \pm SE) in Normal Subjects Given 400 mg Aspartame, 135 mg Saccharin, or Unsweetened Beverage^a

Treatment	AUC (mg/dl · min)	C_{max} (mg/dl)	t_{max} (min)
Aspartame	16,664 \pm 314	96 \pm 2	19 \pm 10
Saccharin	16,179 \pm 191	92 \pm 1	8 \pm 6
Unsweetened	16,213 \pm 174	92 \pm 1	19 \pm 6

^a Adapted from Horwitz *et al.* (1988).

TABLE 2

Plasma Glucose Pharmacokinetics (Mean \pm SE) in Subjects with Type 2 Diabetes Given 400 mg Aspartame, 135 mg Saccharin, or Unsweetened Beverage^a

Treatment	AUC (mg/dl · min)	C _{max} (mg/dl)	t _{max} (min)
Aspartame	34,135 \pm 3720	205 \pm 23	28 \pm 10
Saccharin	32,208 \pm 3767	195 \pm 22	9 \pm 5
Unsweetened	33,860 \pm 4010	205 \pm 24	15 \pm 12

^a Adapted from Horwitz *et al.* (1988).

peppermint flavor/fragrance conditioned stimulus with an unconditioned stimulus of ingestion of glucose (50 g) or aspartame (control). They found no “conditioned” plasma insulin and blood glucose responses and thus no “conditioned” hypoglycemia.

In a randomized, three-way crossover study, Melanson *et al.* (1999) evaluated the effect of carbohydrate, fat, and aspartame in beverages on blood glucose concentrations over a period ranging from 270 to 515 min. They reported that aspartame was followed by blood glucose declines (4 of 10 subjects), increases (2 of 10 subjects), and no changes (4 of 10 subjects), which were related to the sweetness perception of the drinks by subjects and predictive of subsequent intakes. However, given the variability of the data in such a small number of subjects, it is impossible to draw any consistent conclusions regarding whether aspartame had any effect on blood glucose concentrations.

Long-Term Studies with Aspartame in Subjects with Diabetes Mellitus

Stern *et al.* (1976) administered either placebo or aspartame (1800 mg/day in the form of capsules) to Type 2

diabetics for 13 weeks. There was no effect of aspartame on fasting blood glucose concentrations or other clinical laboratory parameters measured. There were also no effects on electrocardiograms or ophthalmologic examinations.

In the study of Tamura *et al.* (1984), aspartame was used as a substitute for sugar in the diets of Type 2 diabetic subjects for 3 months (average intake of 1820 mg/day). There were no changes in glycemic control, including fasting glucose concentrations, glycohemoglobin, and oral glucose tolerance. Okuno *et al.* (1986) studied the effect of a 2-week administration of aspartame (125 mg/day) on fasting blood glucose concentrations and at 1 and 2 h after a 50-g oral glucose load in nine subjects with Type 2 diabetes. The dose of aspartame was equivalent in sweetness to that of the estimated average daily intake of sugar in Japan (20–30 g). There were no significant changes in glucose tolerance tests at the end of the 2 weeks, and no significant changes in fasting or 1- and 2-h postprandial blood glucose concentrations. In addition, blood cholesterol, HDL cholesterol, and triglyceride concentrations were unaffected.

In a randomized, double-blind, placebo-controlled study, Nehrling *et al.* (1985) evaluated subjects with Type 1 and Type 2 diabetes who were given 2700 mg of aspartame ($N = 29$) or placebo ($N = 33$) daily for 18 weeks. The dose of aspartame is equivalent to that in approximately 5–6 liters of beverage sweetened with 100% aspartame per day for an adult. At weeks 9, 17, and 18, there were no differences in fasting or 2-h postprandial blood glucose concentrations or glycohemoglobin concentrations between the aspartame and placebo groups (Fig. 1). The authors concluded that aspartame is safe for use by individuals with diabetes.

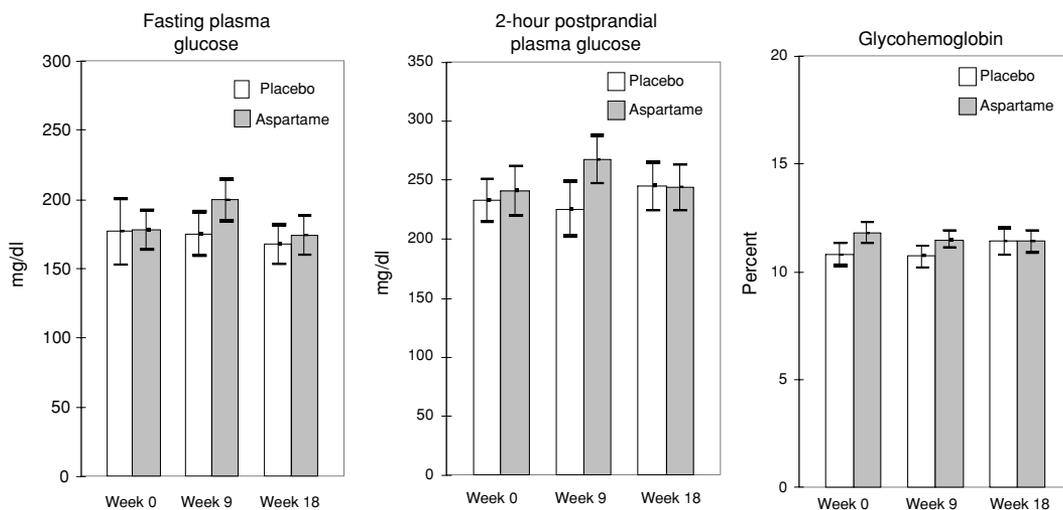


FIG. 1. Long-term aspartame dosing has no effect on fasting or 2-h postprandial glucose (mean \pm SE) concentrations and on glycohemoglobin concentrations (% \pm SE) compared to placebo in Type 1 and Type 2 diabetic subjects. Source: Nehrling *et al.* (1985).

Glucose Metabolism after Exercise

Wouassi *et al.* (1997) compared metabolic and hormonal responses during repeated bouts of brief and intense exercise after administration of glucose or control (aspartame). Venous plasma lactate increased significantly after exercise, but no differences were observed between glucose and aspartame treatments. Initially, blood glucose concentrations decreased but then increased during exercise in both groups. Plasma insulin concentrations decreased in both groups but exhibited a higher peak after glucose treatment than aspartame. Glucagon and epinephrine concentrations did not change significantly in either group.

In Vitro Study with Rat Pancreatic Islet Cells

An *in vitro* study by Malaisse *et al.* (1998) evaluated the effect of different high-intensity sweeteners on insulin release from rat pancreatic islet cells. They found no effect of aspartame but did observe increased insulin release with other high-intensity sweeteners that are known to have bitter components (i.e., saccharin, cyclamate, stevioside, and acesulfame-k).

Other Endocrine Evaluations

Carlson and Shah (1989) evaluated the effect of aspartame (534 mg) and its constituent amino acids aspartic acid (242 mg) and phenylalanine (300 mg and 1000 mg) on serum concentrations of prolactin, cortisol, growth hormone, and insulin, and blood glucose concentrations in healthy individuals. Aspartame (in doses equivalent to that present in about 1 liter of beverage), aspartic acid, or phenylalanine (in equimolar amounts) did not change the magnitude and pattern of secretion of prolactin, cortisol, or growth hormone. The high dose of phenylalanine (1000 mg/kg body wt) did modestly increase serum prolactin concentrations to a level expected after a high-protein mixed meal. There were some minor increases in serum glucose and decreases in serum insulin concentrations in all test groups, which were thought to be related to postprandial alterations from breakfast eaten 3–5 h earlier. Aspartame did not affect glycemic control, as there was no increased activity of insulin counterregulatory hormones (e.g., cortisol and growth hormone).

In a randomized, double-blind study, Melchior *et al.* (1991) gave normal subjects a chocolate drink with sucrose (50 g) or aspartame (80 mg). Plasma glucose concentrations increased significantly after sucrose; they also increased after aspartame but were not significantly different compared to the fasting condition. Plasma insulin concentrations increased after both aspartame and sucrose compared to fasting. Plasma concentrations and AUC of β -endorphin were significantly higher after aspartame compared to sucrose or fasting. The authors suggested a possible cephalic insulin re-

lease effect caused by aspartame, but this finding is not consistent with the results of other studies discussed later (Härtel *et al.*, 1993; Teff *et al.*, 1995; Abdallah *et al.*, 1997).

Nguyen *et al.* (1998) evaluated the effect of single doses of aspartame (250 mg) and glucose (75 g) on calcium and oxalate metabolism in a crossover study in healthy adults. They reported that urinary calcium excretion increased after both aspartame and glucose, which they related to increased calcemia and decreased phosphatemia. Aspartame did not alter plasma concentrations of glucose or insulin or urinary oxalate excretion. However, these findings from a single-dose study are contrary to those observed by Leon *et al.* (1989, 1999), who reported no differences between blood calcium concentrations or urinary calcium excretion after very high doses of aspartame (about 20 times the dose given by Nguyen *et al.*) given daily for 6 months compared to placebo or baseline. The dose of aspartame administered in the study by Leon *et al.* (75 mg/kg body wt/day) is the equivalent of a 70-kg individual consuming the amount of aspartame in over 10 liters of beverage sweetened with 100% aspartame daily for 6 months.

Cephalic-Phase Insulin Release

It has been suggested that sweetness may induce cephalic-phase insulin release (CPIR), resulting in lowering of blood glucose concentrations with consequent increases in hunger (Rogers *et al.*, 1988). To investigate this, Härtel *et al.* (1993) evaluated five different test solutions (containing aspartame, acesulfame-k, cyclamate, saccharin, or sucrose) and water in a multiple crossover study. Blood was drawn at 0, 5, 10, 15, 30, 60, and 120 min after dosing for plasma insulin and blood glucose determinations. As expected, sucrose resulted in a significant increase in plasma insulin and blood glucose concentrations compared to the high-intensity sweeteners and water, exceeding the normal range between 10 and 30 min. Generally, glucose concentrations were within the normal range, and there were no significant differences in plasma insulin or blood glucose concentrations with the four high-intensity sweeteners compared to water. The authors concluded that there is no cephalic insulin secretion from high-intensity sweeteners.

Teff *et al.* (1995) did a randomized study using 1- and 3-min oral exposures in random order to solutions of aspartame, saccharin, and sucrose and water, which were then expectorated. An apple pie served as a modified sham-feed condition. Blood was drawn at 0 and 1 min, every 2 min for 15 min, and then every 5 min for 15 min. There were no significant increases in plasma insulin concentrations after any of the sweetened solutions, but the apple pie resulted in significant increases in plasma insulin concentrations after both the 1- and the 3-min

exposures. Thus, the taste of sweetened solutions alone was not adequate to stimulate CPIR.

Abdallah *et al.* (1997) reported the results of a randomized, double-blind, placebo-controlled study to evaluate CPIR with oral exposure (sucking on the tongue) to three different tablets: tablets contained 3 g of sucrose, 18 mg of aspartame plus 3 g polydextrose (a nonsweet carbohydrate), or 3 g polydextrose as the placebo. Blood was drawn at 1-min intervals for 45 min before sucking the tablet and for 25 min after the beginning of sucking. Plasma glucose, insulin, and glucagon concentrations were not modified after aspartame or sucrose tablets, suggesting that sweet taste per se was not sufficient for eliciting CPIR.

Sugar and Aspartame in the Diets of Diabetic Subjects

With ongoing interest in nutritional guidelines for diabetics, Colaguri *et al.* (1989) undertook a double-blind, crossover study for 6 weeks on each treatment to evaluate the effect of sucrose vs aspartame on the diets of well-controlled Type 2 diabetic subjects. Sucrose or aspartame, given with each meal and in beverages between meals, had no effect on glycemic control (fasting blood glucose, glycosylated hemoglobin, or insulin concentrations). In addition, there were no effects of either aspartame or sucrose on serum total cholesterol, HDL-cholesterol, or triglyceride concentrations. These authors concluded that aspartame was a suitable sugar substitute for use in diabetics, although sucrose added as a part of the diabetic diet did not adversely affect glycemic control in well-controlled diabetic subjects.

More recently, Tepper *et al.* (1996) evaluated the relationship between sweet taste and dietary intake in subjects with Type 2 diabetes and controls administered sucrose, fructose, and aspartame. Among the various groups, there were no differences in sweet taste perception, pleasantness ratings, daily energy intakes, or macronutrient composition of diets. Diabetic subjects were found to ingest less sucrose but 3.5 times more aspartame compared to controls.

Conclusions

Results of the acute dosing studies reveal that aspartame does not affect the glycemic response in normal or diabetic subjects. In addition, aspartame does not affect metabolic control when administered to diabetic subjects daily for up to 18 weeks. There are also no effects of aspartame on plasma lipid profiles in subjects with diabetes mellitus. Aspartame also had no effect on hormones such as prolactin, growth hormone, and cortisol. Several studies have evaluated the effect of aspartame on cephalic-phase insulin release. The results of these studies demonstrated no cephalic-phase insulin release induced by aspartame.

The American Diabetes Association (2002) has evaluated the use of aspartame in diabetic diets. It found aspartame to be an acceptable sweetener for diabetics and supports the consumption of aspartame at the acceptable daily intake (ADI) in the United States of 50 mg/kg body wt, which approximates the aspartame content in about 6–7 liters of beverage sweetened with 100% aspartame daily for an adult. Actual aspartame consumption, however, is well below the ADI.

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Evaluation of Appetite, Food Intake, and Usefulness of Aspartame in Weight Control

Introduction

Obesity has become a worldwide epidemic. The World Health Organization (WHO) has developed a comprehensive analysis of the problem (WHO, 2000). The primary concern is the health hazard of being overweight or obese and the issues of how best to prevent excessive weight gain and to treat different degrees of overweight and obesity. The prevalence rates for overweight and obese people are different in each region, with the Middle East, Central and Eastern Europe, and North America having higher prevalence rates. In most countries, women show a greater body mass index (BMI in kg/m^2) distribution with higher obesity rates than do men. Obesity is usually now associated with poverty, even in developing countries. Abdominal obesity in adults, with its associated enhanced morbidity, occurs particularly in those who had lower birth weights and early childhood stunting (James *et al.*, 2001). Obese individuals are at an increased risk for the development of a number of serious diseases, including diabetes, hypertension, cardiovascular disease, gallbladder disease, and certain types of cancer (U.S. Department of Health and Human Services, 2001). At the same time, evidence suggests that moderate weight loss (5–15% of body weight) will reduce health risks and medical problems in the majority of obese individuals (Blackburn and Kanders, 1987, 1992; Blackburn, 2001). Thus, the prevention and treatment of obesity remains a significant worldwide public health priority (Khaodhiar and Blackburn, 2001).

Despite many billions of dollars being spent yearly (Begley, 1991) by approximately 23% of men and 40% of women trying to lose weight (Horm and Anderson, 1993), long-term maintenance of weight loss with either conventional or nonconventional treatment remains disappointing with more than 95% of all dieters regaining lost weight within 5 years of treatment (Robinson *et al.*, 1993; Wadden, 1993). Recently, Anderson *et al.* (2001) conducted a meta-analysis of 29 reports of long-term weight-loss maintenance indicating that weight-loss maintenance 4 or 5 years after a structured weight-loss program averages 3.0 kg or 23% of initial weight loss, representing a sustained reduction in body weight of 3.2%. Although success in weight-loss maintenance has improved over the past decade, much more research is required to enable most individuals to sustain the lifestyle changes in physical activity and food choices necessary for successful weight maintenance (Blackburn, 1999).

High-intensity sweeteners, such as aspartame, are not a panacea for weight loss or weight maintenance. However, aspartame can replace the sugar in foods and beverages and thus result in reduced or no calorie

products. Researchers have investigated the use of such nutrient-modified foods to improve the outcome in the treatment of obesity. Such research must be well controlled with careful attention paid to all variables, including caloric intake and level of exercise. Incorporation of such reduced calorie foods and beverages into the diet, as part of a multidisciplinary regimen also including exercise and lifestyle changes, can help motivated individuals to lose or maintain body weight.

Safety of Aspartame in Obese Individuals

As discussed in other sections, aspartame has undergone rigorous safety testing, including comprehensive toxicology studies in animals and studies in humans. In addition, both short-(6 weeks) and long- (21 weeks) term randomized, double-blind, placebo-controlled studies (Hoffman, 1972, 1973) in obese adults have demonstrated the safety of aspartame use by this population. Another randomized, double-blind, placebo-controlled 13-week study of aspartame administered in capsules to obese adolescents and young adults on weight reducing diets also showed no differences in weight change, plasma triglyceride level, serum cholesterol, or parameters of hematologic, hepatic, or renal function (Knopp *et al.*, 1976).

Epidemiological Evaluation of Weight Change and Artificial Sweetener Use

In 1986, Stellman and Garfinkel suggested a relationship between artificial sweetener use and weight change over a 1-year period. This was based on the results of a study using self-reported body weight change and food intake data from a prospective epidemiologic cancer study to determine retrospectively whether there was a relationship between body weight and use of intense sweeteners. The authors concluded that users of artificial sweeteners were significantly more likely than nonusers to gain weight, regardless of initial weight. However, obese users lost significantly more weight than did obese nonusers, a fact often overlooked in discussions of this paper. Further, the authors themselves noted that artificial sweetener users who lost weight or whose weight did not increase could have gained weight had they not consumed the sweeteners (Stellman and Garfinkel, 1988).

This study was criticized (Lavin *et al.*, 1994) for both methodological flaws in experimental design and statistical analysis. The flaws identified included the use of data from an unrelated study for which they were not intended, the failure to correct for bias as a result of convenience sampling, the unvalidated use of data from a homogeneous subpopulation, and the stratification of

subjects by BMI that was determined from outcome data which was gathered near completion of the 10-year retrospective study. In addition, it was inappropriate to compare weight changes in individuals who gained weight vs individuals who lost weight since the outcome of the study, weight change, determined the populations being compared.

Appetite, Hunger, and Food Intake

Blundell and Hill (1986, 1987) suggested that aspartame may stimulate appetite, speculating that the use of aspartame by certain individuals "may lead to a loss of control over appetite . . . , and contribute to disordered patterns of eating." However, these studies did not measure the effect of aspartame on food intake, an important omission as food intake can be dissociated from reported hunger (Mattes, 1990). In a later study, researchers from this same laboratory (Rogers *et al.*, 1988) reported that subjects had an increase in hunger after drinking water or solutions of saccharin, acesulfame-K, and aspartame compared to glucose solution. However, actual food intake after the high-intensity sweeteners was not significantly different when compared with drinking water alone. They speculated that this "paradoxical" effect of aspartame on appetite may be due to cephalic-phase insulin release (CPIR). However, as discussed in detail under the section "Endocrine Evaluations with Aspartame," aspartame does not cause CPIR.

In another study from this same laboratory, Rogers *et al.* (1990) reported that aspartame ingested without tasting (in capsule form) inhibited hunger and food intake. They speculated that this was due to a postingestive inhibitory effect of aspartame on appetite. Black *et al.* (1993) reported the results of an experiment comparing the effects of different volumes of beverage, beverages with aspartame in solution, and beverages with aspartame in capsules on appetite and food intake. In contrast to the report of Rogers *et al.* (1990), Black and co-workers (1993) reported that aspartame in capsules had no effect on appetite. Furthermore, they concluded that any appetite reduction after consumption of an aspartame-sweetened beverage is likely due to the volume of the drink and not the aspartame.

Many other studies, using various research paradigms, have been done to evaluate the effect of aspartame on hunger, appetite and food intake. Replacing sucrose with aspartame in foods or beverages has not been shown to increase food intake or hunger in children (Anderson *et al.*, 1989; Birch *et al.*, 1989) and has not been shown to increase food intake in normal weight (Blundell and Hill, 1987; Rolls *et al.*, 1989, 1990; Black *et al.*, 1991; Canty and Chan, 1991; Drewnowski *et al.*, 1994a,b) or in overweight men and women (Rodin, 1990; Drewnowski, 1994b). It should be noted that all of these studies reported either unchanged or reduced motiva-

tion to eat regardless of whether the aspartame was delivered in a solid or liquid medium. Recently, Wilson (2000) compared the effect of plain milk, sucrose-sweetened milk, and aspartame-sweetened milk on mealtime caloric intake in young children. Children consumed more sweetened milk than plain milk. However, the authors found that young children do not reduce caloric intake at a meal to compensate for the extra calories resulting from sucrose-sweetened milk whereas aspartame increased milk consumption without providing the extra calories of sucrose-sweetened milk. Studies on aspartame, appetite, and food intake have been reviewed in detail by Rolls (1991), Renwick (1994), Drewnowski (1995), and Rolls and Shide (1996). As Rolls and Shide (1996) concluded, "From evaluation of the available data, there is no consistent nor compelling evidence that the intense sweetener aspartame increases food intake or body weight."

In a series of inpatient studies done in metabolic wards, Porikos *et al.* (1977, 1982) and Porikos and Pi-Sunyer (1984) investigated food intake following covert caloric dilution with aspartame to evaluate whether normal weight and obese individuals would maintain their usual caloric intake in response to covert caloric dilution of their diet. In one study with obese subjects (Porikos *et al.*, 1977) where aspartame replaced sucrose (approximately 25% of total calories), energy intake decreased significantly to 77% of baseline intake during the first 3 days and remained at 86% of baseline intake during the second 3 days. In normal weight volunteers (Porikos *et al.*, 1982), there was a significant reduction in caloric intake during a 12-day period on an aspartame-sweetened diet. Intake was lowest in the first 3-day period (76% of baseline) with stabilization of intake at 85% of baseline during the subsequent nine days of the caloric diluted diet. In the third study of both obese and normal weight subjects (Porikos and Pi-Sunyer, 1984), the investigators again observed a 15% reduction in energy intake when aspartame was substituted for sucrose in the diet. While these data suggest that there is no short-term physiologic drive to replace all calories removed through the use of aspartame, the long-term effects are unclear. In addition, these studies have been criticized because the baseline diet encouraged overeating and weight gain and did not reflect the subjects' normal diet.

In an outpatient study, Tordoff and Alleva (1990) investigated the effect of adding aspartame in a blinded manner to a normal diet in free-living normal weight subjects. During two 3-week experimental periods, subjects received four bottles of beverage (40 oz. or 1135 g) sweetened with either aspartame or high-fructose corn syrup per day. During one 3-week control period, they received no experimental drink. Compared with results during the control period, there was a significant reduction in caloric intake in both females and males and decreased body weight of males during the period

when aspartame-sweetened soda was consumed. However, the high-fructose corn syrup treatment led to a significant increase in caloric intake and body weight in both sexes.

More recently, Astrup *et al.* (2002) reported that large amounts of sucrose (mean 152 g/day) added to the diets of overweight subjects for 10 weeks resulted in increases in total energy intake, body weight, fat mass, and blood pressure. In contrast, body weight, fat mass, and blood pressure decreased in overweight subjects who were supplemented with artificial sweeteners.

Weight Control

To evaluate the effect of aspartame on short-term control of body weight in obese subjects, Kanders *et al.* (1988, 1996) recruited 59 obese (37 kg/m²) men and women to participate in a pilot study with a 12-week multidisciplinary diet program. Subjects were randomly assigned to consume a balanced deficit diet (1000 ± 200 kcal/day) with or without aspartame-containing products. Although not statistically significant, women in the aspartame group (*N* = 24) lost 3.7 pounds more than women in the no-aspartame group (*N* = 21). Men showed the opposite trend, with those in the no-aspartame group (*N* = 7) losing about 4 lb more than those in the aspartame group (*N* = 4).

Forty-six of these subjects (11 males and 35 females) participated in a 1-year follow-up study. Increased levels of physical activity, increased consumption of aspartame, and decreased desire for sweets were associated with maintenance of weight loss at the 1-year follow-up. In male subjects, aspartame intake at the end of follow-up was associated with better weight maintenance. Although definitive conclusions could not be drawn due to the small sample size, aspartame consumption did not cause weight gain and may be beneficial in promoting weight loss and maintenance when used as part of a multidisciplinary weight control program.

As a follow-up to this pilot study, Blackburn *et al.* (1997) conducted a randomized, controlled, prospective clinical study to investigate whether the addition of aspartame to a multidisciplinary weight control program would improve weight loss and long-term control of body weight in obese women. Initially, 168 obese women aged 20 to 60 years were placed on a nutrient-balanced deficit diet (1000 ± 200 kcal/day) for 3 weeks. At the end of this period, 163 subjects were instructed to continue the balanced deficit diet and were randomly assigned either to consume aspartame-sweetened foods and beverages during the remaining 16 weeks of active weight loss phase or to avoid such products. During the 1-year weight maintenance phase and 2-year follow-up periods, participants were encouraged to continue to consume or avoid aspartame-containing products according to their original group assignment.

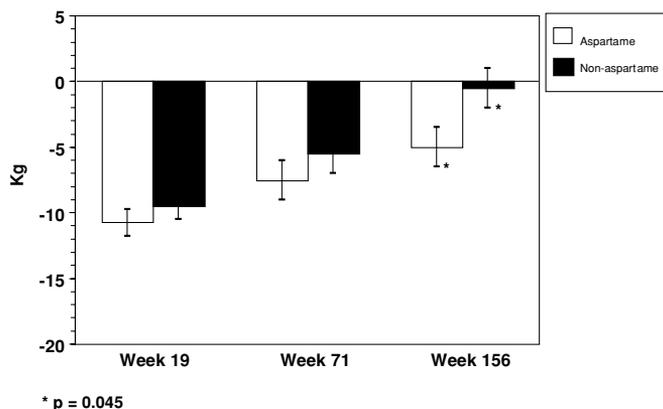


FIG. 1. Mean change in body weight at weeks 19, 71, and 156 compared to baseline with aspartame and no-aspartame treatment groups. Adapted from Kanders *et al.* (1996).

During the active weight loss period, all subjects also attended weekly 1-h sessions where they were instructed on behavioral and lifestyle strategies to facilitate weight loss. During 12-month maintenance and the 19-month follow-up, the groups met monthly. Throughout the study, regular exercise, mainly in the form of walking, was strongly encouraged. Parameters evaluated at baseline, 19, 71, and 156 weeks included body weight, aspartame intake, exercise level, and subjective ratings of hunger, desire for sweets, and eating control.

One-hundred thirty-six subjects completed the active weight loss phase; 125 subjects completed the maintenance phase; and 86 subjects completed the follow-up phase. Subjects in both treatment groups lost a mean of approximately 10% of body weight (10 kg) during the 19 weeks of active weight loss (Fig. 1). Among subjects in the aspartame group, aspartame consumption was positively associated with weight loss. The desire for sweets decreased significantly in the aspartame group but not in the no-aspartame group; hunger did not differ significantly from baseline in either treatment group, but eating control increased significantly in both treatment groups. In the maintenance phase (weeks 19–71), hunger and desire for sweets remained unchanged within both treatment groups. Eating control decreased significantly in both maintenance and follow-up phases in both groups, suggesting more uncontrolled eating during maintenance and follow-up.

At the end of the maintenance phase (Week 71), subjects in the aspartame group experienced a 3.1% mean weight regain, and those in the no-aspartame group regained a mean of 4.9%. By the end of the follow-up phase (Week 156), subjects in the aspartame group had regained an additional 2.4%, with a net weight loss from baseline of 5.1%. In contrast, subjects in the no-aspartame group had a gain of 5.4%, with a net weight loss of 0.3% from baseline (Fig. 1). Significant predictors of better weight control from baseline to Week 156 included increased exercise, increased self-reported

eating control, and initial treatment group assignment, where aspartame group subjects had an advantage over the no-aspartame group subjects. The authors concluded that aspartame, as part of a multidisciplinary weight control program, may facilitate weight control.

Conclusions

Nutritional surveys estimate that added sugars account for approximately 15–20% of calories in the American diet. High-intensity sweeteners such as aspartame can provide sweetness and palatability without adding calories and may facilitate control of body weight in individuals who are motivated to do so. The few studies that indicated an increased motivation to eat following the consumption of aspartame were not replicated by a number of other studies. Further, inpatient investigations of nondieting obese and normal weight individuals have demonstrated incomplete caloric compensation after the covert replacement of sucrose with aspartame.

Participation in a multidisciplinary weight control program that included the use of aspartame-sweetened foods and beverages facilitated weight loss, as well as long-term maintenance of a reduced body weight. Taken together, these results suggest that aspartame, when incorporated into a multidisciplinary weight management program also including exercise and behavior modification, may aid in the long-term control of body weight.

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Conclusions

Aspartame is unique among high-intensity sweeteners because it is metabolized by digestive esterases and peptidases to three common dietary constituents—the amino acids, aspartic acid and phenylalanine, and methanol. These constituents are used by the body in the same metabolic pathways as when they are also derived from foods, such as meat, milk, fruits, and vegetables. Further, these constituents are derived from common foods in much larger amounts than from aspartame in foods and beverages. For example, a glass of no-fat milk provides about 6 times more phenylalanine and 13 times more aspartic acid and a glass of tomato juice provides about 6 times more methanol than an equivalent volume of beverage sweetened 100% with aspartame. Much of the scientific research, both before and after regulatory approval, focused on the safety of these components.

Prior to the regulatory approvals of aspartame, a comprehensive battery of toxicology studies was done in animals to evaluate acute, subchronic, and long-term toxicity, carcinogenicity, genetic toxicity, and reproductive toxicity and teratogenicity. These studies demonstrated that aspartame is not toxic, carcinogenic, mutagenic, or teratogenic and has no effects on reproduction or development. Other studies demonstrated aspartame has no effects on the central nervous system, gastrointestinal tract, endocrine system, and reproductive system or on postnatal developmental in infant primates. In addition, a number of metabolism and tolerance studies were done in humans with healthy adults, infants, children and adolescents; PKU heterozygotes; obese individuals; and individuals with diabetes. This comprehensive database supporting the safety of aspartame has been evaluated by regulatory agencies and expert committees around the world and determined to demonstrate aspartame's safety for its intended use as a sweetener. During the past 20 years since regulatory approvals, aspartame has been safely consumed by hundreds of millions of people worldwide.

Since the marketing of aspartame, a number of scientific issues were raised and thoroughly addressed through animal and human studies. As before approval, these issues centered largely on hypothetical toxicity of its three metabolic components when given in extremely large doses and continued to include potential neurotoxicity (excitotoxicity) of aspartic acid, potential effects of phenylalanine on brain function, and potential toxicity of the methanol metabolite formate. These issues were resolved to the satisfaction of worldwide regulatory agencies prior to approval of aspartame. Nonetheless, additional studies after approval have included evaluation of additional preclinical safety paradigms; aspartame intake relative to the acceptable daily intake; metabolism; aspartate and excito-

toxicity; phenylalanine and neurochemistry; methanol; postmarketing surveillance of anecdotal reports of health effects; allegations of headaches, seizures, effects on behavior, cognitive function, and mood, as well as allergic-type reactions and brain tumors; safety of aspartame use by potentially sensitive populations; effects on endocrine function; and effects on appetite, food intake, and weight control. The weight of the evidence from these additional studies further confirmed the results of the earlier studies and expanded the database on aspartame safety. The conclusions of these extensive evaluations are summarized below.

Preclinical Safety Evaluation of Aspartame

The definitive preclinical studies done with aspartame demonstrated that aspartame is not toxic, carcinogenic, mutagenic, or teratogenic and has no effects on reproduction. In addition, there were no effects of aspartame on the central nervous system, gastrointestinal tract, endocrine system, or reproductive system. From the results of the toxicology studies, a no-observed-effect level of at least 4000 mg/kg body wt and an ADI of 40 mg/kg body wt were established for aspartame in Europe and Canada and by JECFA. When aspartame was first approved in the United States, the FDA established an ADI of 20 mg/kg body wt for aspartame but later increased the ADI to 50 mg/kg body wt/day based on the availability of human data.

Since regulatory approvals of aspartame in the early 1980s, a number of additional animal safety studies were completed. In no cases have subsequent studies altered original determinations of safety established by regulatory agencies as part of the approval process. Regulatory agencies regularly review preclinical safety data; these bodies evaluate safety data according to defined standards and internationally accepted biological and statistical criteria. Critics who fault aspartame safety data, or those who postulate deficiencies in the aspartame database, rarely have a background in evaluating safety data and seldom apply internationally accepted criteria in formulating their critiques.

Intake of Aspartame vs The Acceptable Daily Intake

Aspartame intake has been evaluated in the United States and several European countries, as well as Canada, Australia, and Brazil. Average daily aspartame intake at the 90th percentile ("eaters" only) in the U.S. general population in the last survey was 3 mg/kg body wt. Consumption by 2- to 5-year-old children in these surveys ranged from about 2.5 to 5 mg/kg body wt/day. Although methodologies differed among the evaluations from various countries, aspartame intake in these surveys is remarkably consistent and is

well below the U.S. ADI of 50 mg/kg body wt/day and that of 40 mg/kg body wt/day for much of the rest of the world.

Metabolism of Aspartame

The metabolism of aspartame has been evaluated both with doses relevant to actual consumption and at very large doses highly unlikely ever to be consumed. These studies were done in healthy adults, 1-year-old children, adult PKU heterozygotes, PKU homozygous individuals, and individuals with sensitivity to MSG and have included various testing paradigms—acute bolus dosing, repeated dosing, dosing with meals, and dosing with monosodium glutamate (MSG). In addition, Phe/LNAA has been calculated in a number of these studies; this ratio is thought to be predictive of the amount of phenylalanine that enters the brain. Together, these studies demonstrate that amounts of aspartame far exceeding 90th percentile consumption result in concentrations of aspartame's constituents— aspartate, phenylalanine, and methanol—that are considered safe.

Aspartate and Excitotoxicity

There was speculation early on that the aspartate constituent of aspartame, especially when consumed with foods containing MSG, may result in an increase in the combined plasma concentrations of aspartate and glutamate which might pose a risk of focal brain lesions. Focal brain lesions are reproducible after extremely high doses of glutamate or aspartate in neonatal rodents, but whether these lesions occur in primates remains very controversial in that several investigators have failed to replicate the single report of this effect in infant primates. However, numerous metabolism studies in humans have demonstrated that it is impossible for a human ever to consume enough aspartame in products, even when combined with MSG, to raise plasma concentrations of aspartate or glutamate or glutamate plus aspartate to those concentrations associated with toxicity in neonatal rodents.

Phenylalanine and Neurochemistry

Speculation has been made that the phenylalanine component of aspartame may alter brain function and, consequently, result in effects such as headaches, seizures, and behavioral, mood, or cognitive changes. Further, it was hypothesized that aspartame, as a source of phenylalanine without the other large neutral amino acids that compete for transport across the blood–brain barrier, would selectively increase entry of phenylalanine into the brain by increasing the Phe/LNAA ratio. It was further speculated that any such increase in entry of phenylalanine, accompanied by decreased entry of tyrosine and tryptophan, might

result in disturbances in brain neurotransmitter concentrations. However, numerous metabolism studies in adult humans, infants, and PKU heterozygotes demonstrate that consumption of aspartame in amounts well above typical consumption levels results in plasma phenylalanine concentrations that are safe.

Numerous studies using enormous doses of aspartame were done in laboratory animals to evaluate the hypothesis that very large doses of aspartame might alter brain concentrations of neurotransmitters and their metabolites. The results of these studies do not support this hypothesis, but rather show no reproducible effects on various brain neurotransmitter systems after enormous doses of aspartame. Other studies show no effects of very large doses of aspartame on the central release of neurotransmitters and receptor kinetics. Despite large increases in plasma Phe/LNAA, there are no consistent effects on brain chemistry, even with doses of aspartame up to 1000 times the 90th percentile human consumption. Thus, it is not unexpected that the data are overwhelming that aspartame does not affect brain chemistry or function (e.g., headaches, seizures, or behavior, cognition, and mood).

Safety of Methanol from Aspartame and the Diet

The typical diet, including fruits and vegetables, juices, wine, and other alcoholic beverages, provides greater amounts of methanol than does aspartame in products. Nonetheless, the safety of methanol derived from aspartame was extensively evaluated in both tolerance and metabolism studies in humans. A dose of at least 50 mg/kg body wt aspartame must be ingested to detect any increase in blood methanol concentrations. No increases in blood formate concentrations, the toxic metabolite of methanol, are seen even after bolus doses of aspartame up to 200 mg/kg body wt and after long-term daily doses of 75 mg/kg body wt/day. These doses of aspartame are the equivalent of an adult consuming about 28 liters of beverage sweetened 100% with aspartame as a bolus or over 10 liters of beverage daily for 6 months, respectively. It is clear that the capacity for methanol metabolism in humans is far greater than estimated dietary intake of methanol from aspartame. Thus, it is impossible for a human ever to consume enough aspartame in products to raise blood formate concentrations to levels associated with toxicity.

Postmarketing Surveillance: Evaluation of Anecdotal Reports of Health Effects

As a first for a food additive, a postmarketing surveillance program was implemented to document and evaluate anecdotal reports from consumers of adverse health effects allegedly associated with aspartame. The vast majority of these reports were from U.S. consumers; however, reports from other countries were also

included. Epidemiologists at CDC and FDA concluded that the reports represented symptoms that were generally mild and that are common in the general population. Although it is not possible to determine a cause-and-effect relationship based on such information, it was utilized to guide additional research efforts. As a result, a number of studies were done in the postmarketing period to evaluate these allegations, including headaches, seizures, behavior, cognition, and mood effects, and allergic-type reactions. Other studies were done to evaluate issues regarding potential sensitivity in certain subpopulations. The results of these studies demonstrated that, when evaluated under randomized, double-blind, placebo-controlled conditions with appropriate statistical analysis, aspartame is not associated with adverse health effects.

Evaluation of Aspartame and Headaches

Headache is the most common symptom anecdotally associated with aspartame. A randomized, double-blind, placebo-controlled study with individuals who were convinced that aspartame caused their headaches was done in a clinical research unit where confounding variables that are present in an outpatient setting could be controlled. The authors of this study found no difference between the occurrence of headache after aspartame and placebo. In addition, there is no association between aspartame and headache in numerous other human studies that have been done with aspartame, including a high-dose, long-term study. The few published reports suggesting an association between aspartame and headache are either anecdotal in nature, case reports, or outpatient studies that had statistical issues that prevented drawing any valid conclusions. Thus, the weight of the scientific data demonstrates that aspartame does not cause headache.

Evaluation of Aspartame and Seizures and Electroencephalograms (EEGs)

Numerous studies using various animal models of epilepsy and several human studies evaluated allegations that aspartame is associated with seizures and changes on EEGs. The studies in animals used doses of aspartame up to 3000 mg/kg body wt or 1000 times 90th percentile daily intake in the United States (equivalent to an adult human consuming about 420 liters of a beverage sweetened with 100% aspartame as a single bolus). Although some studies using very large bolus doses of aspartame in the pentylenetetrazol (PTZ) model in rats indicate an effect of aspartame, these results are not reproducible in other laboratories and appear to be largely specific to species and study conditions. One study in children with absence seizures appears to suggest an effect of aspartame on the time spent per hour in spike-wave discharges, however, the study lacked a true placebo and had serious methodological issues because

of an inadequate baseline period. Studies with individuals who were convinced that aspartame was responsible for their seizures and in children with seizure disorders, along with studies in healthy adults and PKU heterozygotes, demonstrate no effects of aspartame on clinical seizures or EEGs. Thus, the evidence is compelling that aspartame, even in amounts greatly exceeding 90th percentile intake, is not a proconvulsant and has no effect on EEGs.

Evaluation of Aspartame and Behavior, Cognitive Function, and Mood

A number of studies using various testing paradigms and very large doses of aspartame (hundreds of times 90th percentile average daily aspartame consumption in the United States) have been done to evaluate behavior, learning, and memory in laboratory animals. The only observed effects are seen after parenteral (generally ip) dosing, a route of administration not relevant to the consumption of aspartame in the diet. Taken as a whole, the data from animal studies do not provide support for purported effects of aspartame on behavior following human consumption. Further, studies in humans, including healthy adults and PKU heterozygous adults, healthy children, and children with ADD, ADHD, or "sugar sensitivity," have evaluated various measures of behavior, cognitive performance, and mood and have consistently demonstrated no effects of aspartame. Thus, aspartame, even when given in doses that result in significant increases in Phe/LNAA, has no effect on behavior, cognition, or mood.

Evaluation of Aspartame and Allergic-Type Reactions

Several single case reports suggested that aspartame is associated with allergic-type reactions. However, several clinical studies have been done to evaluate this issue. In two of these studies with subjects reporting allergic-type reactions associated with aspartame, extensive recruitment efforts over several years failed to identify more than about 10–20 subjects to enroll in each of these studies. One of these studies also included evaluation of allergic-type reactions with diketopiperazine and β -aspartame, which are conversion products of aspartame. Evaluations of allegations of allergic-type reactions and aspartame in controlled clinical studies have consistently demonstrated that aspartame is not an allergen. Further, animal and *in vitro* studies demonstrate that aspartame is not a direct mast cell or basophil secretagogue and has no effect on inflammation parameters such as carrageenan-induced paw edema, connective tissue formation, and adjuvant arthritis. Thus, the weight of evidence demonstrates that aspartame is not associated with allergic-type reactions in experimental models or humans.

Evaluation of Aspartame and Brain Tumors

In the late 1970s, Olney performed a post hoc analysis on results from long-term bioassays in rats to suggest that aspartame may be associated with brain tumors. In one study he combined independent treatment groups and then claimed his results showed a dose-response relationship between aspartame and brain tumors. Further, he asserted that another long-term bioassay in rats was invalid because he claimed the incidence of brain tumors in controls exceeded what he considered to be appropriate for historical controls. Regardless of Olney's claims, scientists in regulatory agencies and expert committees around the world, including the U.S. FDA, the Canadian Health Protection Branch, the UK Committee on Toxicity, and the Joint FAO/WHO Expert Committee on Food Additives, who have evaluated these data according to standard and internationally accepted criteria, have concluded that aspartame is not a carcinogen.

In 1996, Olney *et al.* again asserted that aspartame may be associated with brain tumors, repeating earlier claims regarding the rat studies and further asserting the marketing of aspartame was associated with an apparent increase in incidence of human brain tumors in the United States. This newer assertion was based on their analysis of selected data from the U.S. SEER tumor database from the U.S. NCI. However, NCI has concluded that Olney's claims were not valid, as have regulatory agencies in the United States, the European Union, the United Kingdom, and Australia/New Zealand. The scientific consensus is that there is no association between aspartame consumption and brain tumors.

Use of Aspartame by Potentially Sensitive Populations

Several subpopulations of individuals, including PKU heterozygotes, individuals with depression, individuals with dizziness, individuals with Parkinson's disease, individuals with renal disease, and individuals with liver disease, have been studied because of the possibility that these populations may be potentially sensitive to aspartame. Studies in depressed patients and patients with dizziness did not have an adequate number of subjects completed; thus it is not possible to draw any valid conclusions from these studies. Studies in PKU heterozygotes demonstrate that aspartame, even in amounts well above average 90th percentile consumption levels, results in plasma phenylalanine concentrations that are considered safe. Further, large doses of aspartame given over 12 weeks have no effect on cognitive function or EEGs compared to placebo in these individuals. No effects of aspartame are seen in

individuals with Parkinson's disease, whose symptoms and responsiveness to levodopa are sensitive to fluctuations in plasma amino acid concentrations. Finally, aspartame has been demonstrated to be safe for individuals with renal disease and individuals with liver disease.

Endocrine Evaluations with Aspartame

Individuals with diabetes are likely to be enthusiastic consumers of aspartame because of their dietary restrictions. Thus, studies were done to evaluate the effect of aspartame on plasma glucose and insulin concentrations. From the results of the acute dosing studies, aspartame does not affect the glycemic response in healthy individuals or diabetic subjects. In addition, aspartame does not affect glycemic control, including glycohemoglobin concentrations, when given to diabetic individuals over 18 weeks. Aspartame also has no effect on other hormones, such as prolactin, growth hormone, and cortisol. Several studies have demonstrated no effect of aspartame on cephalic-phase insulin release, disproving suggestions that aspartame may cause cephalic-phase insulin release and thereby affect hunger. Thus, aspartame has been found to be an acceptable sweetener for diabetic individuals by The American Diabetes Association.

Evaluation of Appetite, Food Intake, and Usefulness of Aspartame in Weight Control

High-intensity sweeteners such as aspartame can provide sweetness and palatability without adding calories to the diet and thus may facilitate control of body weight in motivated individuals. Although a few studies indicated an apparent increase in hunger or appetite following aspartame, a number of other studies have not replicated these results. In addition, investigations of nondieting obese and normal weight individuals in metabolic wards demonstrate incomplete caloric compensation after the covert replacement of sucrose with aspartame. Taken together, the results of the research demonstrate no compelling evidence that aspartame has an effect on appetite and food intake. In a prospective weight loss study, a multidisciplinary weight control program that included the use of aspartame-sweetened foods and beverages facilitated weight loss, as well as long-term maintenance of a reduced body weight. These results suggest that aspartame, when incorporated into a multidisciplinary weight management program also including exercise and behavior modification, may aid in the long-term control of body weight.

Summary

The extensive research done with aspartame overwhelmingly demonstrates its safety. In addition, aspartame has been consumed by hundreds of millions of people around the world over the past 20 years, representing billions of man-years of safe exposure. Nonetheless, the safety of aspartame continues to be challenged by individuals who either ignore the data or are not familiar with the totality of the data. The allegations raised regarding aspartame by a few individuals have not been based on controlled scientific studies. Rather, they have been largely based on anecdote, personal opinion, conjecture and hypothesis. Further, the few

studies suggestive of an apparent effect of aspartame are not reproducible by others, are done with dosage routes of administration (e.g., ip or *in vitro*) not relevant to aspartame consumption, or are so flawed from a methodological or statistical standpoint that valid conclusions cannot be drawn.

The scientific evaluation of aspartame's safety has extended well beyond standard safety testing for food additives. When the safety data for aspartame are evaluated as a whole, the weight of scientific evidence is clear that aspartame is safe for its intended uses, and there are no unresolved questions regarding its safety.

Appendix

List of Acronyms and Abbreviations

5-HIAA	5-hydroxyindole acetic acid	LiCl	lithium chloride
α -AP	α -aspartylphenylalanine	LNAA	large neutral amino acid
ADA	American Diabetes Association	MAFF	UK Ministry of Agriculture, Fisheries & Food
ADD	attention deficit disorder	MEDLINE	database from U.S. National Library of Medicine
ADHD	attention deficit hyperactivity disorder	MRCA	MRCA Information Services, Northbrook, Illinois
ADI	acceptable daily intake	MRI	magnetic resonance imaging
ANZFA	Australia/New Zealand Food Authority	MSG	monosodium glutamate
ARMS	Adverse Reaction Monitoring System	NCI	National Cancer Institute
ATP	adenosine triphosphate	NMDA	<i>N</i> -methyl-D-aspartate
AUC	area under the concentration curve	PBOI	Public Board of Inquiry
β -AP	β -aspartylphenylalanine	Phe	phenylalanine
CDC	Centers for Disease Control	Phe/LNAA	plasma ratio of phenylalanine to the other large neutral amino acids
CFSAN	Center for Food Safety and Applied Nutrition	PKU	phenylketonuria
C_{\max}	maximum plasma concentration	PKUH	heterozygotes for phenylketonuria
CNS	central nervous system	PME	phenylalanine methyl ester
CPD	cumulative cell population doubling	POMS	profile of mood states
CPIR	cephalic-phase insulin release	PSE	portal systemic index
CSFII	Continuing Survey of Food Intakes by Individuals	PTZ	pentylenetetrazol
CT	computed tomography	RAST	radioallergosorbent test
DKP	diketopiperazine	SCF	EU Scientific Committee for Food
DNA	deoxyribonucleic acid	SEER	U.S. Surveillance, Epidemiology and End Results
DOPAC	dihydroxyphenylacetic acid	SPARTANS	cognitive battery of aviation-related information processing tasks
DSST	Digit Symbol Substitution Test	SSS	Stanford sleepiness scale
EEG	electroencephalogram	t_{\max}	time to mean peak concentration
EU	European Union	THF	tetrahydrofolate
FAO	Food and Agriculture Organization	Tyr	tyrosine
FDA	U.S. Food and Drug Administration	UAREP	Universities Associated for Research and Education in Pathology
FETAX	Frog Embryo Teratogenesis Assay	USDA	United States Department of Agriculture
GABA	γ -aminobutyric acid	VAMS	Visual analogue mood scales
GEPR	genetically epilepsy-prone rats	VCR	video cassette recorder
GI	gastrointestinal	WHO	World Health Organization
HDL	high-density lipoprotein	Wt	Weight
HVA	homovanillic acid		
IgE	immunoglobulin E		
ip	intraperitoneal		
JECFA	Joint FAO/WHO Expert Committee on Food Additives		