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Food and Drug Administration

[Docket No. 75F-0355]

Aspartame; Commissioner's Final Decision

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Food and Drug Administration

[Docket No. 75F-0355]

Aspartame: Commissioner's Final Decision

AGENCY: Food and Drug Administration.

ACTION: Notice; final decision following a hearing before a public board of inquiry.

SUMMARY: The Commissioner of Food and Drugs is issuing his Final Decision concerning the food additive petition for the nutritive sweetener aspartame submitted by G. D. Searle & Co. The Commissioner has determined that aspartame has been shown to be safe for its proposed uses as a food additive and is approving the petition. Specifically, the Commissioner finds that the available data establish that there is a reasonable certainty that human consumption of aspartame: (1) At projected consumption levels, will not pose a risk of brain damage resulting in mental retardation, endocrine disfunction, or both; and (2) will not cause brain tumors. Accordingly, the Initial Decision of the Public Board of Inquiry is affirmed in part and reversed in part, as modified and supplemented herein.

EFFECTIVE DATE: October 22, 1981.

ADDRESS: The transcript of the hearing, evidence submitted, and all other documents listed in this decision may be seen in the Dockets Management Branch (formerly the Hearing Clerk's office) (HFA-305), Food and Drug Administration, Rm. 4-62, 5600 Fishers Lane, Rockville, MD 20857, from 9 a.m. to 4 p.m., Monday through Friday.

FOR FURTHER INFORMATION CONTACT: Ted Herman, Regulations Policy Staff (HFC-10), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, 301-443-3480.

SUPPLEMENTARY INFORMATION: The purpose of this proceeding has been to decide whether aspartame has been shown to be safe under section 409 of the Federal Food, Drug, and Cosmetic Act (the act) (21 U.S.C. 348).

I. Introduction

A. The Product

Aspartame [L-aspartyl-L-phenylalanine methyl ester] is a dipeptide composed primarily of two amino acids, phenylalanine and aspartic acid. These, along with other amino acids, are normal constituents of protein foods consumed as part of any healthful diet. When phenylalanine and aspartic acid are combined in a certain way to form aspartame, they produce an intensely sweet tasting substance, approximately 180 times as sweet as sucrose. Accordingly, as a sugar substitute, the amount of aspartame needed to produce the same degree of sweetness is substantially reduced, as will be the resulting calories.

Aspartame does break down spontaneously to diketopiperazine (DKP). If present in large amounts, DKP can make aspartame lose its sweetness. Under the uses approved in this decision, however, DKP normally comprises less than 2% of the final aspartame product which does not detract from the product's sweet tastes.

B. Historical Chronology

1. *Initial FDA Approval.* Aspartame was discovered and formulated by G. D. Searle & Co. (Searle), Skokie, Ill. As the law requires for all food additives, Searle petitioned the Food and Drug Administration (FDA) for approval to market aspartame as a sweetening agent in certain foods (38 FR 5921, March 5, 1973). Searle's petition contained voluminous amounts of data purporting to establish the safety of aspartame.

On July 26, 1974, FDA approved Searle's petition and issued a regulation authorizing the use of aspartame in certain foods and for certain technological purposes (39 FR 27317; correction notice, 39 FR 34520, Sept. 26, 1974). That regulation became codified in 21 CFR 172.804. Aspartame was specifically approved for use as a sweetener in the following foods:

a. Dry, free-flowing sugar substitutes for table use (not to include use in cooking) in package units, not to exceed the sweetening equivalent of 2 teaspoonfuls of sugar.

b. Sugar substitute tablets for sweetening hot beverages, including coffee and tea.

c. Cold breakfast cereals.

d. Chewing gum.

e. Dry bases for: (i) Beverages; (ii) instant coffee and tea; (iii) gelatins, puddings and fillings; and (iv) dairy products and toppings. In chewing gum, aspartame was also approved for use as a flavor enhancer in addition to use as a sweetener.

This approval had three conditions regarding final product labeling. First, the label of any food containing aspartame was required to bear the following statement:

"PHENYLKETONURICS: CONTAINS PHENYLALANINE." This requirement was designed to alert persons who, because of specific health reasons, need to restrict carefully their phenylalanine intake (just as diabetics need to restrict their sugar intake). The second condition for approval was that when aspartame was to be used as a tabletop sweetener, its label was required to bear instructions not to use aspartame in cooking or baking. This is because aspartame breaks down to DKP when exposed to prolonged heat, with a consequent loss of sweetness. Finally, if a food containing aspartame purported to be, or was represented, for special dietary uses, as might be expected of a low calorie product, it was required to be labeled in compliance with FDA's special dietary foods regulations (21 CFR Part 105).

2. *Objections to FDA Approval.* As permitted by law (21 U.S.C. 348(f)(1)), two parties formally objected to the regulation on safety grounds and requested a formal evidentiary hearing (21 CFR Part 12). These parties were John W. Olney, M.D., and jointly, James S. Turner, Esq., and Label, Inc. (Legal Action for Buyers' Education and Labeling). Dr. Olney, then as Associate Professor of Psychiatry at the Washington University School of Medicine, St. Louis, Mo. (now Professsor), had performed research in animals regarding the toxic effects on the brain of certain Amino acids, including aspartic acid. Mr. Turner, a lawyer, represented himself and Label, Inc., a consumer-oriented group concerned about the regulation of chemicals in foods. Both parties objected primarily to the use of aspartame by children, asserting that the product might cause brain damage resulting in mental retardation, endocrine dysfunction, or both.

These parties later waived their right to a formal evidentiary hearing conditioned upon the establishment of a Public Board of Inquiry ("Board") consisting of three qualified scientists from outside the agency (21 CFR Part 13). This would be the first time FDA had ever used this alternative procedure. Searle agreed to delay marketing of aspartame temporarily, pending resolution of the safety questions.

3. *Audit of Searle Studies.* Before a Board could be convened, preliminary results from an audit of the records of certain animal studies conducted by or for Searle, including studies on aspartame, indicated a need for a comprehensive review of the authenticity of the aspartame research data. As a result, pursuant to 21 U.S.C. 348(e), FDA formally stayed the regulation authorizing the marketing of aspartame (40 FR 56907, Dec. 5, 1975).

With the knowledge and approval of Searle, the aspartame data in 15 pivotal studies were thoroughly audited to determine their authenticity. Three of these studies were audited by FDA and 12 by the Universities Associated for Research and Education in Pathology, Inc. (UAREP). This was a massive undertaking and took over two years to complete. UAREP concluded that the studies were authentic and, on December 13, 1978, submitted its 1,062 page report to FDA (Vols. 110, 111 and 112).[1] The agency agreed with UAREP that those 12 studies, as well as the three studies which it had reviewed, were indeed authentic. FDA then turned its attention to arranging the public hearing.

4. Establishment of Public Board of Inquiry

Dr. Olney, Searle, and FDA's Bureau of Foods (the Bureau) all submitted nominees for Board membership to then Acting Commissioner Sherwin Gardner who chose the following panel: Walle J. H. Nauta, M.D., Ph. D., Institute Professor, Department of Psychology and Brain Science, Massachusetts Institute of Technology; Peter J. Lampert, M.D., Professor and Chairman, Department of Pathology, University of California (San Diego); and Vernon R. Young, Ph. D., Professor of Nutritional Biochemistry, Department of Nutrition and Food Science, Massachusetts Institute of Technology. Dr. Nauta was named chairman.

As the issues for the hearing were being framed, Dr. Olney raised an additional concern about aspartame's potential to cause brain tumors. Although the Bureau disagreed with Dr. Olney's assessment, then Commissioner Kennedy agreed to add this issue to the hearing agenda (see letter to Dr. Olney, dated November 17, 1978, Vol. 120 [correspondence filed chronologically]).

On June 1, 1979, FDA announced the establishment of the Public Board of Inquiry to help resolve the issues surrounding the proposed marketing of aspartame (44 FR 31716). These issues were defined, in relevant part, as follows:

1. * * * whether the ingestion of aspartame, either aalone or together with glutamate, poses a risk of contributing to mental retardation, brain damage, or undesireable effects on neuroendocrine regulatory systems * * *

2. * * * whether the ingestion of aspartame may induce brain neoplasms (tumors) in the rat * * *

3. Based on answers to the above questions,

(a) Should aspartame be allowed for use in foods, or, instead should approval of aspartame be withdrawn?

(b) If aspartame is allowed for use in foods, i.e., if its approval is not withdrawn, what conditions of use and labeling and label statements should be required, if any?

(44 FR at 31717)

In the Federal Register of January 14, 1980, FDA announced the time and place of the hearing (45 FR 2908). The Board heard 3 full days of testimony, primarily from Dr. Olney and representatives from the Bureau of Foods and Searle.[2] The hearing dates were January 30 and 31 and February 1, 1980. Post-hearing briefs and/or rebuttal statements were filed by Dr. Olney, the Bureau, and Searle.

5. *The Board's Decision.* On October 1, 1980, the Board issued its decision. The Board agreed with the Bureau and Searle on the first issue, finding that aspartame consumption would not pose an increased risk of brain damage, resulting in mental retardation, endocrine dysfunction, or both. However, the Board agreed with Dr. Olney on the second issue, finding that the available data on laboratory rats did not rule out the possibility of aspartame's causing brain tumors, and that, indeed, the evidence suggested that aspartame might induce brain tumors. Accordingly, the Board concluded that aspartame should not be approved for marketing until further animal testing was conducted to resolve the brain tumor issue. Because of the Board's finding on the brain tumor issue, the Board withdrew approval of Searle's food additive petition and, after vacating the stay on the aspartame regulation (21 CFR 172.804), revoked that regulation in its entirety.

Dr. Olney, Searle and the Bureau all filed detailed exceptions to those portions of the Board's decision in which the Board disagreed with their respective positions (21 CFR 12.125(a)) [3] (hereafter "Exceptions"). Mr. Turner also filed exceptions, objecting to the scope of evidence considered by the Board. Searle and the Bureau each filed replies to both Dr. Olney's and Mr. Turner's exceptions (21 CFR 12.125(c)) (hereafter "Replies"). Under the established time frames, the administrative record closed on January 29, 1981, thus making the issue ripe for the final agency decision

II. Statutory Requirements for Approval of a Food Additive Petition

Section 409 of the act (21 U.S.C. 348), sets forth the statutory requirements for approval of a food additive [4] petition. With the enactment of the Food Additives Amendment in 1958, Congress established a premarket approval system whereby the company seeking to market a food additive must first obtain approval from the FDA.[5] Through this mechanism Congress sought to shield the public from unsafe or potentially unsafe products.

Section 409(c)(3) of the act, 21 U.S.C. 348(c)(3), directs FDA not to approve a food additive petition:

* * * if a fair evaluation of the data before the Secretary [6]—

(A) fails to establish that the proposed use of the food additive, under the conditions of use to be specified in the regulation, will be safe: * * *

This provision in the law, known as the "general safety clause," is thoroughly analyzed in the Commissioner's Decision on Cyclamate (Cyclamate Decision) (45 FR 61474, 61476–77, Sept. 16, 1980). Two points of that discussion warrant repeating here.

First, by requiring that the data "establish" safety, Congress clearly placed the burden of proving safety on the sponsor of a food additive petition, in this case Searle. FDA does not have to prove that the product is unsafe. This distinction is very important because it is possible that the data may fall in the "grey area" where the food additive has not been shown convincingly either to be safe or unsafe. In such a situation further testing may be necessary to resolve the issue. This was the agency's position on cyclamate (45 FR at 61477, col. 3). Similarly, Dr. Olney and Mr. Turner contend that the data on aspartame fall into this "grey area" which would require further testing before marketing.

The second essential point in interpreting the general safety clause is the meaning of the term "safe."

[1] All citations to materials in the administrative record refer to the filing system in FDA's Dockets Management Branch (HFA–305).

[2] Mr. Turner made only brief presentations (see Tr./II/pages 187–200 and Tr./III/pages 237–39). Two additional hearing participants, Richard J. Wurtman, M.D., and Lloyd J. Filer, Jr., M.D., also addressed the Board, as did two consultants to the Board, William Nyhan, M.D. and Milton Brightman, M.D.

[3] Although the Board's hearing procedures are set out in 21 CFR Part 13, procedures following issuance of the Board's decision are determined by Subparts G and H of 21 CFR Part 12. See 21 CFR 12.32(f)(3).

[4] The term "food additive" is defined in 21 U.S.C. 321(s). There is no question that aspartame is a food additive.

[5] Any product containing an unapproved food additive is automatically deemed adulterated therefore unlawful. 21 U.S.C. 348(a).

[6] This decision has been delegated to the FDA Commissioner, 21 CFR 5.10(a)(1) (formerly section 5.1(a)(1)) and is not subject to the Secretary's reservation of authority under 5 CFR 5.11 or Executive Order 12291 because decisions on food additives are subject to 5 U.S.C. 556 and 557 (46 FR 13193, Feb. 19, 1981, and 46 FR 26052, May 11, 1981).

Although not defined in the statute itself, FDA regulations clearly reflect the legislative history by stating that safety means:

> * * * there is a *reasonable certainty* in the minds of competent scientists that the substance is not harmful under the intended conditions of use.

21 CFR 170.3(i) (emphasis added). Congressional reports show that the legislators were particularly impressed by expert testimony emphasizing the impossibility of providing, within the bounds of scientific knowledge, the absolute harmlessness of any chemical substance. H.R. Report 2284, 85th Cong., 2d Sess., pp. 4–5, 1958; Senate Report 2422, reprinted in (1958) U.S. Code Cong. and Admin. News 5301.[7] Congress therefore advocated the more realistic, yet still rigorous, standard of reasonable certainty of no harm, later embodied in FDA's regulation quoted above.

The statute leaves the methods and criteria for interpreting data up to the discretion and expertise of the agency. Congress did, however, direct FDA to consider the following three factors:

> (A) The probable consumption of the additive and of any substance formed in or on food because of the use of the additive;
>
> (B) The cumulative effect of such additive in the diet of man or animals, taking into account any chemically or pharmacologically related substance or substances in such diet; and
>
> (C) Safety factors which in the opinion of experts qualified by scientific training and experience to evaluate the safety of food additives are generally recognized as appropriate for the use of animal experimentation data.

21 U.S.C. 348(c)(5). In the case of aspartame, the product's mass marketing potential and expected consumption by persons of all ages, especially children, are aspects that have been considered in the safety evaluation.

The general safety clause applies to all types of health risks. For example, the provision was recently applied to both carcinogenicity and mutagenicity (45 FR 61474). *Cf. Ethyl Corp.* v. *E.P.A.*, 541 F. 2d 1 (D.C. Cir.) (en banc), *cert. denied*, 426 U.S. 941 (1976) (analogous statute applied to lead poisoning).

Food additives presenting health risks may be divided into two categories for safety evaluation purposes: (1) Those which are safe at or below certain levels but unsafe at other, higher levels; and (2) those which may be unsafe at any level. The analysis for these two categories is necessarily different. For example, the first issue in this proceeding, relating to possible "brain damage" (toxicity), concerns the former category whereby aspartame may be marketed so long as the projected consumption levels fall sufficiently below the estimated toxic threshold. In contrast, with respect to the second issue in this proceeding, relating to possible "brain tumors" (carcinogenicity), aspartame must be shown to a reasonable certainty not to cause brain tumors at all, for food additives producing carcinogenic effects at any level are deemed to be unsafe *per se*.[8]

In summary, the general safety clause places on Searle the burden of proving that the data in the administrative record establish that there is a reasonable certainty that aspartame will not be harmful under the prescribed conditions of use. Only if Searle meets this burden can the food additive petition be approved.

III. Summary of Decision[9]

The purpose of this proceeding has been to determine whether aspartame has been shown to be safe under Section 409 of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. 348. The legal standard for approving Searle's Food additive petition is whether there is a reasonable certainty in the minds of component scientists that aspartame will not be harmful to the public under its proposed conditions of use. What is required of the agency, therefore, is the conscientious exercise of principled, scientific judgment. As Commissioner of Food and Drugs, my responsibility is to review the evidence and evaluate it fairly, state my reasons for crediting or not crediting certain evidence, weight all the evidence, apply the correct legal standard, and decide.

This I have done, and I have concluded that aspartame has been shown to be safe for its proposed uses. My reasons for this conclusion, detailed in Sections IV–VII below, may be summarized as follows.

A. The Brain Damage Issues

The first set of objections to the aspartame regulation concerned two distinct types of brain damage, one associated with each of aspartame's two amino acid components, phenylalanine and aspartic acid. The Board disagreed with these objections and found aspartame to be safe in terms of potential brain damage.

Two points stand out which require affirming the Board's decision on these brain damage issues. One is the enormously large amounts of aspartame that a normal person would need to consume before reaching even a cautiously estimated toxic threshold. The second is the remarkably low amount of amino acid intake which would result, from even the 99th percentile of estimated aspartame consumption, in relation to the prevalence of these same amino acids in common protein foods.

1. *Phenylalanine:* The concern that has been raised over aspartame's phenylalanine (PHE) moiety is that sustained plasma-PHE levels above a certain toxic threshold may cause mental retardation, especially in the unborn fetus, similar to that resulting from phenylketonuria (PKU).

The toxic threshold for plasma-PHE levels is 100 micromoles per deciliter (μmol/dl) for normal persons, including infants, and 50 μmol/dl for pregnant women in order to protect their fetuses.[10] Normal plasma PHE levels range from 6 to 12 μmol/dl. Ingestion in a *single sitting* by an adult of a loading dose of aspartame, comparable to the 99th percentile of projected aspartame consumption for an *entire day,* caused plasma PHE levels to rise from a fasting level of 6 μmol/dl to a peak of only 11 μmol/dl, still within the normal range after eating and nowhere close to the toxic threshold. In clinical testing it took approximately six times that amount to induce plasma-PHE rise to the 50 μmol/dl level. For a 60 kilogram adult (132 pounds), this corresponds to 600 aspartame tablets or 24 liters (approximately 6½ gallons) of aspartame-sweetened beverage consumed in a single sitting. Such an

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[7] These Congressional reports are quoted at length in 45 FR at 61477, col. 1.

[8] *But see Monsanto* v. *Kennedy*, 613 F. 2d 947, 955 (D.C. Cir. 1979), which indicated that the agency has discretion to determine that the quantity of a potentially carcinogenic substance found in a food "may be so negligible as to present no public health or safety concerns * * *"

It should also be noted that another portion of section 409, dealing specifically with carcinogenicity (the so-called Delaney Clause), is not applicable in this proceeding. That provision prohibits the approval of any food additive petition where the additive has been shown conclusively to be carcinogenic (see generally, Cyclamate Decision, 45 FR at 61476, col. 3). As noted above, however, those opposing approval of aspartame do so on the grounds that the data on aspartame fall into the "grey area" (i.e., safety has not been demonstrated), not that aspartame is conclusively carcinogenic.

[9] This summary was made publicly available on July 15, 1981 as a vehicle for announcing the decision as soon as possible, without awaiting publication of the entire Final Decision in the Federal Register. Minor changes or deletions have been made to conform to or avoid unnecessary redundancy with other portions of this Final Decision, or to correct typographical errors.

[10] The difference for fetuses is that the placenta maintains a 1:2 ratio gradient between the maternal and fetal circulation in the plasma PHE concentrations (Board's Decision at 13). Thus, a plasma PHE level of 50 μmol/dl in an expectant mother creates a plasma PHE level of 100 μmol/dl for her fetus.

enormous intake at one time, let alone continuously over a sustained period, is inconceivable. Thus, it appears that consumption of aspartame in reasonable amounts, or even in unreasonable but physically possible amounts, will not cause the type of brain damage of concern here (see generally, Board's Decision at 13–15).

I also agree with the Board's conclusion that the marketing of aspartame will not create any additional risk to PKU children not on a restricted diet, individuals heterozygous for PKU, undetected cases of PKU, or pregnant women with the special condition of hyperphenylalanemia (see Board's Decision at 15–20).

Another way to consider the phenylalanine issue is to compare the projected aspartame consumption to the amount of phenylalanine present in common protein products. For example, consumption of aspartame at the projected 99th percentile level (34 mg/kg/day) would increase the normal overall PHE daily intake by only about 6 percent. Even consumption of aspartame at the unlikely level of three times that projected 99th percentile level would increase the normal overall PHE daily intake by only 15 to 20%, still within expected, normal variations in protein consumption (Board's Decision at 20–21). Thus, from the standpoint of phenylalanine intake, aspartame appears to present no greater hazard than common protein rich foods considered essential for proper nutrition.

2. *Aspartic Acid:* The concern raised over increased aspartic acid (ASP) consumption stems from animal studies showing that extremely high doses of ASP, glutamic acid (GLU), and other "excitatory" amino acids can cause focal brain lesions, primarily in areas of the brain that regulate the endocrine system. With two important differences described below, the analysis parallels that in the phenylalanine section in terms of first setting a toxic threshold and then determining whether the projected consumption of aspartame will keep plasma levels sufficiently below that threshold. The first difference is that it is the *combined* plasma ASP+GLU levels which must be scrutinized, both because administration of either GLU or ASP increases plasma levels of both amino acids, and because the two amino acids are equipotent and mutually additive in producing the lesion (see Board's Decision at 22–23). Glutamic acid is prevalent in the food supply, often as the food additive monosodium glutamate (MSG). The second difference is the scientific belief that a *single surge* of plasma GLU+ASP levels above the toxic threshold can cause brain damage, unlike the case with phenylalanine toxicity where a sustained high plasma level is needed.

The Board established the plasma GLU+ASP level of 100 μmol/dl as the toxic threshold for risk assessment purposes. This was an estimate, and an extremely conservative one, based on experimental findings in the most sensitive species at the most sensitive age (the infant mouse). Even with this cautious approach, the data clearly establish safety for anticipated aspartame consumption.[11]

Clinical testing in adults using high aspartame doses (equivalent to 1½ times those at the 99th percentile of projected daily consumption), administered at a single sitting, showed no significant rise in either the plasma ASP or GLU concentrations. Even with an enormous aspartame dose (equivalent to six times that at the 99th percentile of projected daily consumption, or 600 to 800 aspartame tablets) administered at a single sitting, the plasma GLU+ASP level rose from 2.7 μmol/dl to only 7 μmol/dl, still within the normal range found after eating. A further study using 1 year old infants showed similar minor rises in plasma ASP+GLU levels. Finally, other studies in adults showed that ingestion of aspartame (equivalent to the 99th percentile of projected daily consumption) did not further increase elevations of plasma GLU+ASP levels caused by the administration of very high doses of MSG alone (see generally, Board's Decision at 32–33).[12]

The Board also addressed the risk to the PKU heterozygote, the nursing infant, and the unborn fetus, and concluded that these groups were at least as safely protected as normal adults or children (*id.* at 33–34). I agree with these conclusions also.

An additional point worth noting is that the plasma ASP+GLU levels that were observed were short-lived, receding to their baseline value after three hours. Thus, as the Board explained, "repeat-doses of the same enormous magnitude, when spaced 3 hours apart, are unlikely to escalate the GLU+ASP concentration much beyond the level induced by the first dose" (*id.* at 37).

Finally, as was the case the phenylalanine component, the ratio of projected aspartic acid consumption resulting from aspartame to that derived from a normal diet is quite small. For example, in the age group of most concern, young children, consumption of aspartame at the 99th percentile level of projected consumption (34 mg/kg/day) will only increase total aspartic acid consumption by approximately 4%, clearly an insignificant amount.

The conclusion compelled by these findings is that the addition of aspartame to the diet, in any conceivable amount (far beyond the upper projected consumption levels), will not cause focal brain lesions of the type alleged by the objectors to the aspartame regulation.

B. The Brain Tumor Issue

This was the issue on which the Board disagreed with the Bureau of Foods and concluded that further testing was necessary before aspartame could be marketed. With due respect to the Board, I agree with the Bureau of Foods that the data presented at the hearing establish that there is a reasonable certainty that aspartame does not cause brain tumors in laboratory rats. This conclusion is confirmed by additional evidence submitted after the Board issued its decision.

1. *Spontaneous incidence rate of brain tumors.* The most controversial issue at the hearing was whether a significant disparity existed between the brain tumor incidence rates as reported in the Searle studies in a certain strain of rat and the spontaneous incidence rate (or background rate) for brain tumors in this strain as reported in the scientific literature. The Board found that such a disparity existed, and that the disparity was so great as to preclude the key Searle rat studies (E–33/34 and E–70) from providing adequate evidence of aspartame's safety (Board's Decision at 43–45). The Bureau of Foods disagreed with the Board, believing that reliable data in the record established a spectrum of reported spontaneous brain tumor incidences that encompassed the rates reported in the Searle studies.

I agree with the Bureau's assessment of the background rate for brain tumors in the pertinent strain of rats. Although the Board placed considerable weight on published studies reporting spontaneous brain tumor incidence rates of less than 1% (.09%, .6%, and .7%), these studies all had some flaws and, in addition, must be supplemented by other data presented at the hearing reporting higher

[11] In contrast to the analysis in the phenylalanine section, the above analysis does not set the toxic threshold for pregnant women (for protection of the fetus) as half that for normal individuals because the placenta forms an effective barrier against the transfer of both ASP and GLU to the fetus (Board's Decision at 34).

[12] Dr. Olney has asserted that an additional study in children is necessary to measure the effects of aspartame administered in conjunction with MSG. For the reasons discussed in Section IV(C)(3)(d) below, I do not believe such an additional study is necessary.

spontaneous incidences (e.g., 2.2% and 3.2%) which are consistent with those in the Searle studies. One reason for giving weight to these studies reporting higher incidences is that the chances are considerably greater that additional tumors may have been missed in the low-incidence studies than that tumors were included by mistake or misdiagnosed in the high-incidence ones.[13]

Of special significance is the reference reporting an incidence of 2.2%. These data were collected by the National Cancer Institute (NCI) from its carcinogenesis bioassay program. The participating organizations were NCI and Hazelton Laboratories, the same laboratory used by Searle for its key aspartame rat studies. Moreover, the rats used for the NCI data were all control animals of the same strain and commercial source used by Searle, and the size of NCI's sample population was nearly identical to the control groups (combined) in Searle's rat studies. The reported spontaneous incidence rates were also nearly identical: 2.2% (8/368) for the NCI data and approximately 2% (7/356) for the combined control groups in the Searle studies.

2. *Comparison with concurrent controls.* Given the consistency between the control incidence rates in the Searle studies and the background rate, I find that Searle's studies should be evaluated primarily by comparing the aspartame-treated animals to their concurrent controls. Using these comparisons, as analyzed by the Bureau of Foods, I find that Searle studies E-70 and E-33/34 both are negative studies.

It is undisputed by the hearing participants that the E-70 study is a negative study when the treatment groups are compared to the concurrent controls. The only controversy lies in the E-33/34 study, where the Board found a possible dose response and accelerated tumor onset, both potential indicators of carcinogenicity. The finding of the dose response, however, is largely dependent on a single, very early-occurring, unusual tumor (a medulloblastoma), which was probably not aspartame related, and the finding of accelerated tumor onset was based in part on factual errors. For these reasons, as detailed in Section V below, I agree with the Bureau of Foods that E-33/34 is also a negative study.

Finally, a third long-term study assessing aspartame's carcinogenic potential using a different strain of rat, concluded recently in Japan and submitted into the record after the Board issued its decision, also appears to be negative in terms of brain tumors. Although this study has not been critiqued by the hearing participants, the data on their face provide additional support for my conclusion on this issue.

3. *Conclusion on brain tumors.* The available data, viewed as a whole, establish that aspartame is safe in terms of brain tumors for its proposed uses.

C. Conditions of Use

The labeling conditions set forth in the aspartame regulation (21 CFR 172.804) before it was stayed shall still be required. These include a prominently displayed alert to persons with PKU that the product contains phenylalanine; directions not to use aspartame in cooking or baking because the compound loses its sweetness when exposed to prolonged heat; and labeling in compliance with FDA's special dietary foods regulations (21 CFR Part 105) where appropriate. In addition, because the safety assessment on the brain damage issues is tied closely to projected aspartame consumption levels, as a condition for approval Searle is to monitor the actual use levels of aspartame and to provide such information on aspartame's use to the Bureau of Foods as the Bureau may deem necessary.

D. Concluding Remarks

The safety evaluation of aspartame has been a long and arduous process, spanning the tenure of several FDA Commissioners. Although my conclusion is the same reached by the agency nearly seven years ago, the intervening years have not been without their benefits in terms of the evidence showing the safety of aspartame. Much of the data, especially clinical data, relied upon by Searle at the public hearing, came from studies conducted at the firm's behest during the interim. Also taking place during this period was the detailed independent audit of Searle's preclinical data conducted by the Universities Associated for Research and Education in Pathology, Inc. (UAREP) and the agency. Few compounds have withstood such detailed testing and repeated, close scrutiny, and the process through which aspartame has gone should provide the public with additional confidence of its safety.

The pinnacle of this process was the hearing before the Public Board of Inquiry, the first of its kind to be convened. The scientific issues presented to it were intellectually complex and carried wide ranging public health ramifications. These scientific issues were debated vigorously at the hearing, and the Board performed admirably in maintaining a judicial decorum and in crystalizing its views of the issues in its Initial Decision. I would be remiss if I did not express to each of the Board members the appreciation of both the agency and the public for the invaluable service which they performed.

IV Evidence on the Brain Damage Issues

The first issue at the hearing was as follows:

> The question has been raised whether the ingestion of aspartame, either alone or together with glutamate, poses a risk of contributing to mental retardation, brain damage or undesirable effects on the neuroendocrine regulatory systems. From available evidence, what can be concluded in relation to this question? The objecting parties believe that the ingestion of aspartame, either alone or together with glutamate, does pose a risk of contributing to these effects. The Bureau of Foods believes that the ingestion of aspartame, either alone or together with glutamate, does not pose a risk of contributing to these effects.

(44 FR at 31717). The Board considered this issue in two parts, one relating to the phenylalanine component of aspartame, and one involving the aspartic acid component. Aspartame's two amino acids are each associated with a different kind of brain damage. Only the aspartic acid component interrelates with glutamate. The Board's subdivision is followed in this decision. Before discussing the specific brain damage issues, however, it is necessary to address the projected consumption levels of aspartame.

A. Projected Consumption Levels

Because the non-toxicity of aspartame is based on safe levels of use, the projected estimates of aspartame consumption are central to the safety evaluation. Three methods have been used to arrive at these estimates, each of which attempt to exaggerate projected consumption levels in order to account for potential heavy users of aspartame-sweetened products.

The first method, used by the Bureau of Foods, is to assume that aspartame is substituted for *all sucrose* in the diet of an average 60 kg man. In this situation, aspartame consumption would be approximately 8.3 mg/kg/day (Tr./I/ page 60, line 11—page 61, line 2). Although this figure is based on the needs of an average consumer rather than a "heavy user," this shortcoming is counterbalanced by the assumption that aspartame would replace all sucrose in

[13] This wider spectrum of reported spontaneous incidence rates is further supported by data submitted into the record by Searle and the Bureau of Foods after the Board issued its decision.

the diet, an unlikely event. Moreover, if aspartame is substituted for *all carbohydrates* as well as all sucrose, the average 60 kg man would consume approximately 25 mg/kg of aspartame per day (Tr./I/page 61, lines 7–23). Surely this would appear to be a highly exaggerated figure.

The second set of consumption estimates was based on data generated by the Market Research Corporation of America ("MRCA"), submitted into the administrative record by the General Foods Corporation (Vol. 103). The MRCA has a large computer bank which tabulates actual dietary records kept by 4,000 households (approximately 12,000 individuals) over a 2-week period, staggered throughout the year. These estimates are based on what people in given age brackets actually eat and are broken down into different percentile levels, to account for both the average and heavy users (Vol. 103, pages 1–3; Tr./I/pages 92–93; Tr./III/page 105).

One additional aspect of the MRCA estimates is that the survey covered two groups of products: "Group A" products were those in Searle's current food additive petition; and "Group B" products included seven additional categories for which aspartame has marketing potential, including carbonated soft drinks, probably the largest potential use (Vol. 103, pages 2–3). Inclusion of the Group B categories provides an extra "cushion" for purposes of Searle's current petition.

The survey showed young children (age 2–5) to be the largest consumers in proportion to their body weights. For Groups A and B combined in this age group, the mean potential exposure was 11.1 mg/kg, and the 90th percentile value was 25.0 mg/kg.[14] The "under 2" and "6–12" age groups were the next biggest users, each with mean and 90th percentile aspartame exposure levels of approximately 6 and 16 mg/kg/day, respectively. The other age groups had decreasing exposure with age, with the "25 and older" category having the lowest 90th percentile level of only 5.9 mg/kg of aspartame per day. (See detailed chart in Vol. 103, page 6.) For all age groups, the 99th percentile figure was 34 mg/kg of aspartame on a daily basis (Tr./I/page 93, lines 10–15).

Finally, Searle's chief witness, Dr. Stegink, calculated yet a third set of figures (using a method similar to that employed by the Bureau of Foods) by substituting aspartame for all carbohydrate energy requirements, including those supplied by sucrose. Using a 70 kg adult, Dr. Stegink estimated this maximum aspartame usage to be 23–25 mg/kg/day (Tr./I/page 94, line 1—page 95, line 3). Dr. Stegink also calculated aspartame intake for a 10 kg infant, assuming aspartame was substituted for all energy requirements now supplied by sucrose, and arrived at an estimated intake of 19 mg/kg/day (Tr./I/page 96, line 6—page 97, line 14).[15]

Although these figures are only estimates, the consistency of the figures across different methods adds significantly to their credibility. In order to be as cautious as possible, the Board used the 34 mg/kg/day figure as the benchmark for use in the risk assessment analysis. This was the highest figure obtained from any of the estimates and represented the 99th percentile for all age groups from the MRCA survey. I agree with the Board's use of this 34 mg/kg/day figure. More importantly, as detailed in the following sections, the non-toxicity of aspartame has been clearly demonstrated in all age groups at levels several times this 99th percentile figure.

B. Phenylalanine

As noted in Section III(A)(1) above, the Board concluded that the projected level of aspartame consumption by normal humans "cannot be expected to increase the incidence of that particular form of mental retardation that is associated with sustained elevation of plasma-PHE levels" (Board's Decision at 20). This conclusion also applies to fetuses, infants, and individuals heterozygous for PKU (*id.* at 14–15). For individuals on a PHE restricted diet (i.e., PKU children and pregnant women known to have hyperphenylalanemia)[16] the Board found that the cautionary label proposed by the Bureau of Foods ("Phenylketonurics: Contains Phenylalanine") would sufficiently protect these individuals who are accustomed to controlling carefully their dietary intake of phenylalanine (*id.* at 21). For the "unfortunate case" of the pregnant woman who does not know she has hyperphenylalanemia, or for undetected cases of PKU children, the Board concluded that "the normal food-derived PHE poses a much greater risk to the patient (or the unborn child) than would aspartame, even when consumed in large amounts" (*id.*).

I agree with the Board's conclusions and careful discussion of these complex issues, and therefore adopt the Board's decision as my own. The relevant portion of the Board's decision (pages 11–22) is reproduced in Appendix A to this decision.[17]

Dr. Olney's raised two exceptions of this issue. The first exception relates to the percentage of PKU children who are not diagnosed at birth (Olney's Exceptions at 1). The Board used a figure of 10%, apparently relying on the testimony of Dr. Koch (Tr./II/page 11). Dr. Koch testified that 10% of all PKU cases may be missed "due to the lack of a good quality program" (*id.*). Dr. Olney asserts that these 10% are missed due to the error inherent in the screening method, and that another group of PKU babies (approximately 20% of those afflicted) are missed because they are among groups of infants that had not been screened at all. Dr. Olney adds these two figures and concludes that

[14] 90th percentile means that in 9 out of 10 days an individual will have an intake equal to or less than the mg/kg figure.

[15] The amount of aspartame that would be used in specific products under consideration, as supplied by the General Foods Corporation and Searle, are as follows:

a. *Table Top Sweetener:* 40 mg in a free flowing packet; 20 mg in the tablet. These are equivalent to two and one teaspoons of sugar, respectively.

b. *Dry Beverage Mix (e.g., Kool Aid or Tang):* 120 mg per 8 oz. glass.

c. *Gelatin or Pudding:* 32 mg per serving (half cup).

d. *Whipped Toppings:* 10 mg per serving (two heaping tablespoons).

e. *Breakfast Cereals:* 90 mg per serving (one oz. or one cup).

f. *Chewing Gum:* 8 mg per stick.

(Searle's Post-Hearing Brief, Vol. 155 at 12–13). These figures are in straight milligram amounts, which need to be divided by the weight of the consumer (in kilograms) for comparison to the estimates described above.

[16] Hyperphenylalanemia, as described in more detailed below in relation to Dr. Olney's exceptions, is a condition whereby a person's plasma PHE levels are higher than normal but lower than a person with PKU. Although these individuals are not themselves brain damaged, pregnant women with this condition may give birth to brain damaged babies if they do not keep themselves on a low phenylalanine diet.

[17] One minor modification to the Board's decision is necessary. The change in no way affects the validity of its conclusions. In various places, the Board uses as a benchmark, for comparison purposes, the amount of phenylalanine intake that would be consumed by a 4 ounce hamburger. The phenylalanine content of a 4 ounce hamburger used by the Board (4,000 mg) was based on testimony by one of Searle's witnesses, Dr. Koch (Tr./II/page 14, lines 21–22). The figure, however, does not appear to be correct for a 4 ounce *cooked* hamburger. According to *Geigy Scientific Tables* (7th Ed., Geigy Pharmaceuticals (1970) at 516), based on the percentage of protein in a cooked hamburger (24.2%) and the percentage of that protein composed of phenylalanine (4.2%), the PHE content of a 4 ounce cooked hamburger is approximately 1,150 mg (not 4000 mg). Although estimates of phenylalanine content vary depending on whether the meat is considered as cooked, uncooked or dry weight, the amount of phenylalanine in a cooked hamburger is the most appropriate comparison for these purposes because that is what people actually eat. A similar adjustment should be made for a hot dog. Even with these changes, the upper projected level of aspartame consumption is still low when compared to the amount of phenylalanine which would be derived from a protein-rich meal.

30% of all PKU children in this country remain undiagnosed.

I disagree. Although the exact number or percentage of PKU children who remain undiagnosed *at birth* was subject to some dispute at the hearing (*compare* Tr./I/page 39, lines 1–16 *with* Tr./II/ page 11, lines 1–8), Dr. William Nyhan, a consultant to the Board and an acknowledged expert on PKU, emphasized that nearly all PKU children who are not diagnosed at birth by a routine screening test are nevertheless diagnosed by 8–10 months of age by the classical diagnostic techniques (i.e., due to abnormal development) (Tr./I/page 224, line 9—page 225, line 8). Dr. Nyhan also emphasized that an infant with PKU, whether diagnosed or not, still needs approximately 90 mg/kg of phenylalanine per day as an essential nutritional requirement (Tr./I/page 229, lines 6–10). These nutritional requirements, together with the fact that aspartame is not being approved for infant formulas or infant foods and that PHE levels are not elevated in breast milk, led Dr. Nyhan to conclude, and I agree, that the undiagnosed PKU infant will not be at additional risk by the marketing of aspartame (Tr./I/page 232, line 1—page 233, line 5; *cf.* Tr./II/page 23, lines 14–18). As the Board so correctly stated, an undiagnosed PKU child "is at risk first and foremost by being undiagnosed and hence permitted to consume meals that are standard for normal children" (Board's Decision at 17).

Dr. Olney's second exception concerns a special subcategory of pregnant women who have a condition known as "hyperphenylalanemia" but do not know it (Olney's Exceptions at 1–2). This is an unusual condition which can affect a fetus without affecting the mother. As noted in Section III above, normal plasma PHE levels vary between 6 and 12 μmol/dl. By comparison, brain damage does not result in a normal individual unless the plasma PHE level is sustained at 100 μmol/dl or higher, or in a fetus unless the mother's plasma PHE level is sustained at a level of 50 μmol/dl or higher. Women with hyperphenylalanemia have plasma PHE levels which fluctuate between 25 and 120 μmol/dl. Thus, most of these women are unaffected themselves because their plasma levels are not sustained above the critical 100 μmol/dl level. What is equally clear, however, is that many of these women during pregnancy will have sustained plasma PHE levels at or above the critical 50 μmol/dl figure, thereby giving birth to "brain-damaged children destined to grow up mentally retarded" (Board's Decision at 19). The only remedy to this problem, however, a problem which currently exists whether or not aspartame is marketed, is first to identify the women who have this condition, and then put them on a phenylalanine restricted diet just as one would a child with PKU (*id.*).

For the reasons stated above and in the Board's decision, I find that the data establish that there is a reasonable certainty that the proposed use of aspartame will not cause or aggravate the type of diffuse brain damage associated with sustained high plasma levels of phenylalanine.

C. Aspartic Acid

1. *Issue:* The second toxicity issue before the Board was whether the expected consumption of aspartame, either alone or together with glutamate (i.e., as MSG), poses a risk to humans of causing focal brain lesions (and associated neuroendocrine changes) of the type which has been demonstrated in animals after the administration of these substances. In addressing this issue, three questions must be answered: (1) Based on extrapolation from animal data, what is the toxic threshold, in terms of the plasma levels of aspartic acid (ASP) and glutamic acid (GLU) which likely have to be reached to induce focal brain lesions in man; (2) in what amounts would aspartame have to be consumed (alone or with MSG) by humans to elevate plasma GLU+ASP to this toxic level; and (3) whether the projected consumption of aspartame will be sufficiently below the amount needed to reach this toxic level.

2. *Board's decision.* After considering these questions, the Board concluded that "[e]levations of plasma GLU+ASP concentration even to the lowest level that could be suspected of being neurotoxic (100 μmol/dl) would require in inconceivably high oral aspartame intake," and that "the ingestion of aspartame, either alone or together with glutamate, cannot be expected to increase the incidence of brain damage or dysfunction of neuroendocrine regulatory systems" (Board's Decision at 38, 39).

I agree with the Board's conclusion and thorough analysis of this issue and therefore adopt the Board's discussion as my own, with minor modifications as noted below. The relevant portion of the Board's decision (pp. 22–38) is incorporated here by reference, and is reproduced in Appendix B to this decision. Because Dr. Olney and the Bureau of Foods have taken exception to various portions of the Board's decision, I will address each of those exceptions directly, after placing the issues in context with a brief background discussion.

3. *Analysis*—a. *Background.* The type of brain lesion of concern here is one which has been studied in animals over the past 12 years. It is produced by high doses of glutamate, aspartate (either given as aspartate *per se* or as aspartame), and by other "excitatory" amino acids and their analogs. The lesion primarily consists of dead or dying nerve cells (neurons.[18] The most sensitive region of the brain appears to be the arcuate region of the hypothalamus; other brain regions and the retina are also affected at higher doses. The affected areas of the hypothalamus are involved in endocrine conrol, via the pituitary gland. Indeed, long-lasting endocrine changes have been produced by administration of high doses of MSG to neonatal mice and rats (reviewed in Vol. 126, Tab 67 (Olney, "Excitotoxic Amino Acids: Research Applications and Safety Implications" in Filer, Jr. et al., eds., *Glutamic Acid: Advances in Biochemistry and Physiology*, p. 287+(1979))). Significantly, it is believed that the lesion can be produced by a single surge of plasma GLU/ASP above some toxic threshold.

The total plasma level of GLU+ASP is a more relevant measure than that of either amino acid alone. As noted in Section III(A)(2) above, this is because administration of either GLU or ASP increases plasma levels of both amino acids and because the two amino acids are equipotent and mutually additive in producing the lesion (see Board's Decision at 22–23).

b. *The toxic threshold.* The Board adopted a plasma GLU/ASP level of 100 μmol/dl as the toxic threshold in humans for risk assessment purposes (Board's Decision at 35). This value was taken from studies in infant mice where an oral dose of 500 mg/kg MSG, given by gavage [19] in aqueous solution, caused focal brain lesions in 50% of the animals. This dosage was then shown to result in a plasma GLU level of approximately 100 μmol/dl. As the Board itself noted, this was a conservative estimate (*id.*). I

[18] The affected parts of the neurons are the dendrites and cell bodies, but not axons.

[19] Test compounds are usually administered by either an oral or parenteral route. Compounds administered orally may be given either mixed with the diet or force-fed by different methods, such as a stomach tube (gavage). Parenteral administration involves the injection of the compound under or through the skin. Examples include: subcutaneous injection ("s.c.") meaning beneath the skin; intravenous injection ("i.v.") meaning into a vein; and intraperitoneal injection ("i.p.") meaning into the peritoneal cavity.

agree that the estimate is conservative, for the following reasons:

(1) *Most Sensitive Animal Species:* The threshold value was derived from studies on mice, which appears to be the most sensitive animal species to this type of brain lesion. Rats and guinea pigs are somewhat less sensitive than mice; lesions have not been produced in dogs (Board's Decision at 24; see also Vol. 126, Tab 9 (Heywood and Worden, "Glutamate Toxicity in Laboratory Animals" in Filer, Jr., *supra,* p. 203+)). The susceptibility of primates is controversial. Most of the data in infant monkeys showed that doses of MSG, capable of raising plasma GLU+ASP levels up to 445 μmol/dl, did not cause lesions (Vol. 152 [Reynolds, Section VII and Stegink, Section VI–A at 2 and Table 1]; see also Bureau's Reply at 7, 8), although one report describes lesions in infant monkeys with plasma GLU levels of approximately 120 μmol/dl (Olney, Vol. 141, Tab I–81). (The interpretation of these studies conducted on monkeys is discussed below.)

(2) *Most Sensitive Age.* The threshold value was based on data in infant mice, the most sensitive age of mouse to elevations in plasma GLU/ASP levels. Sensitivity in the mouse rapidly decreases with age, with weanling and adult mice approximately one-fourth and one-sixth as sensitive, resectively, to elevations in plasma GLU/ASP levels than the infant mouse (Vol. 143, Tab 116 [O'Hara and Takasaki, *Toxicology Letter* 4:299 (1979)]).

Though the Board chose a conservative estimate, I agree with the Board that the value of 100 μmol/dl is the appropriate toxic threshold for risk assessment purposes. In the interest of public health protection, a cautious approach is warranted, especially where, as here, the state or our scientific knowledge does not permit a more precise estimate to be made with sufficient confidence (*cf.* 45 FR at 61480, n. 12). Moreover, even while using this extremely conservative estimate, aspartame's safety regarding focal brain lesions has still been clearly demonstrated. This provides additional confidence that the proposed use of aspartame will not be harmful.

The Bureau believes the toxic threshold should be derived from monkey studies, not rodent studies, because the monkey's brain organization and development allegedly make that animal a more relevant model for humans regarding GLU/ ASP induced brain lesions. Based on the available monkey data, the Bureau considers at least 445 μmol/dl to be a more reasonable toxic threshold (see generally, Bureau's Exceptions at 4–6 and Bureau's Reply at 8–11). I disagree with the Bureau. Even assuming that monkeys are a more appropriate model in this instance, the monkey data available on this issue are not entirely consistent with the Bureau's position. Although data from four laboratories involving 50 infant monkeys support the Bureau's position (Reynolds, Vol. 152 at Section VII and Stegink, Vol. 152, Section VI–A at 2 and Table 1), data from a fifth laboratory involving 6 infant monkeys showed brain lesions at plasma GLU levels of approximately 120 μmol/dl (Olney, Vol. 141, Tab I–81). The Board emphasized that the monkey data are "controversial" and found itself "unable to resolve the conflicts that arose over this issue at the public hearing" (Board's Decision at 25). Consistent with its overall cautious approach, however, the Board "accepted" the value of 120 μmol/dl as the critical plasma GLU level in the immature monkey "to remain on the side of safety" (*id.*).

I agree with the Board that the monkey data are controversial and difficult to resolve on the basis of the current record. However, I disagree with the Board to the extent that it is necessary to make even a tentative finding on this issue. Given the fact that a plasma GLU+ASP level of 100 μmol/dl has been established for risk assessment purposes based on the mouse data, and that *all* the monkey data show a higher toxic threshold, it is not necessary for me to make any conclusion regarding the monkey data for purposes of the aspartame proceeding.

c. *Effect of aspartame on plasma GLU/ASP levels in humans.* The Board concluded that the ingestion of aspartame by humans, even in unusually large quantities, did not cause plasma GLU/ASP levels to rise anywhere close to the estimated toxic threshold of 100 μmol/dl (Board's Decision at 36–38). Indeed, the Board cited convincing data showing that plasma GLU/ASP levels in humans receiving unusually large doses of aspartame remained within normal after eating limits (*id.* at 32, 33, referring to Vol. 152 [Stegink VI–A at 7–9 and VI–B at 31]). For example, a loading dose of 200 mg/kg aspartame in the adult (equivalent to 600–800 aspartame tablets) produced a combined plasma GLU+ASP rise from a baseline of 3 μmol/dl to a peak of only 7 μmol/dl (Vol. 115, Section III). In the 8 to 12 month old infant, a loading dose of 100 mg/kg aspartame caused the plasma GLU+ASP level to rise from a baseline of 9 μmol/dl to a peak of only 11 μmol/dl (Vol. 140, Tab 5).[20]

These human studies, as well as the aspartame/MSG interaction study discussed below in relation to Dr. Olney's exceptions, were performed under "higher risk" conditions (regarding rises in plasma GLU/ASP) than those likely to be encountered under actual use, as follows:

(1) *High Doses.* The extremity of the doses used in the two studies described above is particularly impressive. The 99th percentile of projected *daily* consumption for aspartame is 34 mg/kg. In comparison, no marked plasma ASP+GLU rise in adults or infants was seen with doses administered in a *single sitting* equivalent to 6 and 3 times, respectively, that 99 percentile figure.[21]

(2) *Absence of Carbohydrates:* It has been shown that the presence of food, particularly carbohydrates, inhibits the rise in plasma GLU after MSG dosing (Vol. 149, Tab I–99c (Stegink, et al., "Factors Affecting Plasma Glutamate Levels in Normal Adult Subjects" in Filer, Jr., *supra,* page 333+)). Thus, lesions have not been produced in rodents by aspartame or MSG when administered in the diet, even at extremely high doses, presumably because threshold plasma GLU/ASP levels were not reached. In the human plasma level studies, fasting subjects were used, and the vehicle for aspartame administration was either unsweetened Kool-Aid or orange juice. The vehicle for MSG administration in the interaction study discussed below in relation to Dr. Olney's exceptions was a low carbohydrate consomme.

(3) *High Concentration.* Another factor which may affect plasma levels is the concentration of the substance administered. For example, for a given MSG dosage, the greater the MSG concentration, the greater is the plasma GLU level and hypothalamic damage (Vol. 136, Tab 10 [Bizzi, et al., *Toxicology Letter* 1: 123 (1977)]). The aspartame concentrations in the human studies ranged from 0.6–2.8%, while a typical concentration in a presweetened dry beverage mix is 0.05% (Bureau's Reply at 16). Even with these extremely high concentrations, no significant

[20] The Board noted only the plasma ASP (instead of GLU+ASP) level which peaked at 2.0 μmol/dl (Board's Decision at 33).

[21] It should also be mentioned that these slight increases in plasma GLU+ASP levels are short-lived, i.e., the levels returned to baseline within three hours after ingestion of aspartame. Thus, even repetitive ingestion of these high doses, spaced three hours apart, would not be expected to increase plasma levels above the slight increases produced by the first dose (Board's Decision at 37).

elevations in plasma GLU/ASP levels resulted, as noted above.

The Board also cited data showing that aspartame did not potentiate (i.e., augment) the effect on plasma GLU levels induced by MSG. For example, the Board cited a human study showing that aspartame at 23 mg/kg had no effect on the plasma GLU/ASP levels resulting from ingestion of a hamburger-milkshake meal to which 150 mg/kg of MSG had been added; similar results were also found using doses of 34 mg/kg aspartame and 34 mg/kg MSG (Board's Decision at 32, 33, referring to Vol. 152 (Stegink VI–B at 18–22)). Similar results were found in an aspartame-MSG interaction study performed under "higher risk" conditions (Vol. 139, Tab 12) as discussed below.

d. *Dr. Olney's exceptions regarding the effect of aspartame on plasma GLU/ASP levels in humans.* Dr. Olney has taken strong exception to the Board's decision on this focal brain lesion issue (see generally, Vol. 158, Tab 261). His primary concern is that the Board did not address the question of plasma GLU/ASP levels in *children* following the ingestion of *aspartame in conjunction with MSG* under "high-risk" conditions,[22] and that such a study should be performed in view of the fact that children are already exposed to food products containing large amounts of GLU and ASP. To support his position, Dr. Olney cites the following three lines of evidence which were not discussed in the Board's decision.

First, Dr. Olney states that some commercial soup products contain enough added GLU (as MSG) to provide 100 to 130 mg/kg for a young child, and that a similar dose of GLU, when fed in noncarbohydrate solution to human adults, caused a surge of blood GLU/ASP to levels substantially exceeding the 100 μmol/dl level which the Board determined to be the toxic threshold (Olney's Exceptions at 2–3).

This point, however, concerns the risk associated with ingestion of MSG itself which is not at issue here. What is at issue is whether the addition of aspartame to the food supply will increase or potentiate any elevations of plasma GLU/ASP which might be caused by MSG, and, as discussed below and in the Board's decision, the available evidence suggests that it will not.

Dr. Olney next points to a study in human adults, performed under "high risk" conditions regarding plasma GLU/ASP elevations (i.e., fasting subjects, with MSG given in low-carbohydrate consomme and aspartame given in unsweetened Kool-aid) in which, following the addition of both MSG and aspartame, plasma GLU/ASP levels in *some* individuals were observed to be nearly twice as high as those found following the addition of MSG alone (Olney's Exceptions at 3).

The Board's decision did not discuss the study referred to (Vol. 139, Tab 12 and Vol. 152 [Stegink VI–B at 22–25]).[23] However, Dr. Olney's statement that the "glu + aspartame meal caused GLU/ASP blood levels in *some* individuals to rise nearly twice as high as those induced by glu alone" (emphasis added) presents a one-sided view of the data. In this study, the addition of 50 mg/kg MSG alone resulted in a mean elevation in plasma GLU + ASP from a fasting level of 4.4 ± 1.2 μmol/dl to a peak of 21.0 ± 7.1 μmol/dl. The further addition of 34 mg/kg aspartame resulted in a mean level of 25.7 ± 10.5μmol/dl, which was not different, in terms of statistical significance, from the mean level reached with MSG alone (vol. 139, Tab 12 and Vol. 152 (Stegink VI–B at 24)). Singling out those individual subjects who did show an increase in plasma levels (over MSG alone) after receiving both compounds results, in my opinion, in a scientifically incorrect interpretation of the data. Plasma level data such as this almost always show a certain degree variation between subjects and even in the same subject: this is why hypotheses are accepted or rejected using mean values, standard errors, and statistical methods. Based on mean values obtained in this study, aspartame did not have a significant effect. Citing results only for the individuals who fell on one side of the mean severely biases presentation of a study's findings. In fact, there were some subjects in this study which had *lower* GLU + ASP levels with the combination than with MSG alone, but one would *not* want to conclude from this that aspartame antagonizes (i.e., counteracts) the effect of MSG.[24]

Finally, Dr. Olney has cited data showing that a given oral dose of GLU in young animals produces higher plasma GLU/ASP levels than does the same dose on adult animals. He then analogizes to humans and suggests that children will have higher plasma levels than adults after ingestion of aspartame in conjunction with MSG (Olney's Exceptions at 3).

There are two answers to this point. First, the animal data in the literature are inconsistent. Although some studies do show that plasma GLU/ASP levels are higher in immature animals as compared to adult animals given an equal oral load of GLU or ASP (Vol. 126, Tab 15 [Stegink et al., "Comparative Metabolism of Glutamate in the Mouse, Monkey and Man" in Filer, Jr., *supra*, page 85 +]; Vol. 138, Tab 7 (Oppermann and Ranney, *Journal of Environmental Pathology and Toxicology*, 2:987, 1979)], Dr. Olney's contention that this difference in plasma levels is "well established" is, in my view, not correct. In fact, there are some animal studies in the record, not mentioned by Dr. Olney, which do not show this effect (Vol. 123, Tab 14 (O'hara, et al., *Journal of Toxicology Science* 2:281, 1977); Vol. 136, Tab 10 (Bizzi, et al. *Toxicology Letter* 1:123, 1977)).

More importantly, in *human* studies using *aspartame*, it was shown (under "high risk" conditions) that doses of up to 100 mg/kg were handled as well by 8–12 month old infants as by adults (regarding increases in plasma GLU + ASP). This study was cited by the Board, which stated: "This finding appears to refute any suggestion that aspartic acid might be metabolized less efficiently in infants than in adults." (Board's Decision at 33, referring to Vol. 152 (Stegink VI–B at 31)). I agree. There are also other human studies in the record, not mentioned in either the Board's decision or in Dr. Olney's exceptions, showing that infants (including those which were premature or of low birth weight) have the capability to metabolize dietary GLU and ASP as well as adults (cited in Vol. 152 (Stegink VI–B at 25–31)).

I therefore find, after a consideration of Dr. Olney's exceptions and the available data, that the proposed use of aspartame, either alone or together with glutamate, does not pose a risk of focal brain damage in humans. The data is convincing that that plasma GLU/ASP

[22] I.e., by using noncarbohydrate liquid vehicles, which would mimic the animal gavage studies by providing a bolus dose and thus allow for maximal increases in plasma GLU/ASP.

[23] As noted above, the Board did discuss a study in which the effect of aspartame on MSG-induced rise in plasma GLU/ASP was examined in human adults (no effect of aspartame was noted), although this study was not done under "high-risk" conditions, i.e., the vehicle was a hamburger-milkshake meal (Board's Decision at 32–33).

[24] It can of course be argued that aspartame might have a potentiating effect in some individuals, although a more likely explanation, in light of the fact that in other studies aspartame alone at this and higher doses had no effect on plasma GLU/ASP, is that the higher levels seen in some subjects receiving both compounds represents variability at different times in a single subject's response to MSG. Whatever the explanation, however, the conclusion which must be drawn from this study is that aspartame did not cause a statistically significant potentiation of the effect of MSG.

It might also be noted that the highest plasma GLU + ASP level reached by an individual in this study was 39.6 μmol/dl, which is still well below the toxic threshold as determined above.

levels in human adults receiving unusually large doses of aspartame remained within normal after eating limits, far below the lowest level even suspected of being neurotoxic. Moreover, the available data in human infants strongly suggest that this group handles loads of aspartame, GLU and ASP as well as adults. The lack of significant potentiation of the effects of MSG (on plasma GLU/ASP levels) by aspartame, as shown in adults under "high risk" conditions, provides further evidence of aspartame's safety when consumed with MSG. The addition of aspartame to the food supply, therefore, should not create any additional risk of focal brain lesions in children. I find that Dr. Olney's proposed study in children is unnecessary.[25]

e. *Possible adverse effects at subtoxic levels.* Dr. Olney has also cited animal studies (Vol. 125, Tab 66 (Olney, et al., *Brain Research* 112:420, 1976); Vol. 125, Tab 55 (Terry, et al., *Federal Proceedings* 36:364, 1977)) which have purportedly shown that doses of MSG that are subtoxic, i.e., below those needed to produce focal brain lesions, produced acute changes in plasma hormone levels (presumably via excitatory effects on hypothalamic neurons which control pituitary hormone secretion) and that a study with aspartame + MSG, involving the measurement of neuroendocrine function, should be performed in children for this reason also (Olney's Exceptions at 4, 6).

I disagree. As stated in the Board's decision (pp. 30–31), the hormone level changes noted in these studies were within the range of normal fluctuation, and may "have reflected no more than a normal circadian or ultradian periodicity of (hormone) release," and two other research groups were not able to replicate these findings (Vol. 125, Tab 68 (Yonetani and Matsuzawa, *Toxicology Letter* 1:207, 1977); Vol. 137, Tab 25 (Nemeroff et al., *Brain Research* 156:198, 1978)). Moreover, there was no proof in the "positive" studies that the doses used (1000 mg/kg s.c. or i.p. in adult rats) did not cause hypothalamic lesions. The Board thus concluded that "endocrine disorders are induced by MSG only when this substance is administered in amounts large enough to cause identifiable hypothalamic lesions" (Board's Decision at 31). More importantly, no endocrine toxicity due to *aspartame* at subneurotoxic doses has been reported in animals.

I therefore conclude that, as with the issue of focal brain lesions, aspartame would not pose an additional risk to children of neuroendocrine changes, and that Dr. Olney's proposed study is unnecessary.

f. *Other exceptions.* (1) The Board noted that the record contained one apparent exception to its general statement that no lesions had been observed in animals as a result of the voluntary consumption of GLU or ASP by mixing the test compound with the animal's regular food. The one exception occurred in a study in which 10 weanling mice were offered concentrated solutions of either GLU or GLU + ASP + aspartame after having been deprived of water overnight. All 10 mice developed lesions (Board's Decision at 27, referring to Olney, Vol. 157, Tab 205).

The Bureau takes exception to categorizing this experiment as a valid model for voluntary dietary consumption (Bureau's Exceptions at 6–9), and I agree with the Bureau. These animals were water-deprived, and they drank a small amount of highly concentrated solution over a short period of time at doses known to induce lesions in weanling mice when administered by gavage. Thus, the experimental design was essentially no different from the previous gavage studies.[26]

Finally, although it may be argued that under some conditions human voluntary consumption of aspartame may mimic gavage dosing (i.e., individuals may drink large amounts of aspartame-sweetened beverage at one sitting), human studies performed under these conditions showed that plasma GLU/ASP levels were not substantially increased (Vol. 140, Tab 5 and Vol. 115, Section III).

(2) The Bureau has also taken exception to one statement, apparently made by the Board in passing, which may require clarification. The statement appears on page 29 of the Board's decision where, after discussing neuroendocrine disorders induced in rodents by *subcutaneous* injections of MSG, the Board suggested that "* * * It seems reasonable to assume that in the same species * * * administration of aspartame *by gavage* * * * (at a dose containing an equivalent amount of aspartic acid) * * * would have similar endocrine consequences" (emphasis added). The Bureau's concern is that the route of administration might significantly affect the plasma levels, and that dosing orally by gavage is likely to require a higher dose to produce the same effects as would a subcutaneous dose (Bureau's Exceptions at 9–10).

I agree with the Bureau that some clarification of the Board's statement would be helpful. The critical value is not the dose used, but the plasma GLU + ASP level reached after administration of that dose. Because it is possible that different routes of administration may affect resulting plasma levels, it would be necessary to test the Board's hypothesis which, as the Board itself noted, had not yet been done (Board's Decision at 29). I emphasize, however, that this point is raised for clarification purposes and in no way changes the Board's decision.

4. *Conclusion.* For the reasons stated above and in the Board's decision, I find that there is a reasonable certainty that the proposed use of aspartame, either alone or together with glutamate, will not cause focal brain lesions in man or other adverse effects on the neuroendocrine system.

V Evidence on the Brain Tumor Issue

A. Introduction.

1. *Issue Presented.* The second major issue at the hearing was defined as follows:

> The question has been raised whether the ingestion of aspartame may induce brain neoplasms in the rat. From available evidence, what can be concluded in relation to this question? The objecting parties believe that available evidence suggests, without adequately ruling out, a possible association between aspartame ingestion and an increased incidence of brain neoplasms in the rat. The Bureau of Foods believes that available evidence does not show that ingestion of aspartame results in an increased incidence of brain neoplasms in the rat.

(44 FR at 31717). In layman's terms, neoplasms are tumors. Thus, stated in the context of the legal standard, the issue is whether the data establish that there is a reasonable certainty that aspartame does not cause brain tumors in laboratory rats.

2. *Background.* The brain tumor issue falls under the general category of

[25] Dr. Olney has requested, in the event aspartame is approved for marketing without requiring his proposed study in children, that the Board and myself jointly sign an affidavit stating that we consider such a study to be safe (Olney's Exceptions at 6). I decline to do so. My sole responsibility in this proceeding is to render a decision on the issues raised at the hearing, as defined in the July 1, 1979 Federal Register notice. This I have done. As for the Board, its responsibilities were fulfilled with the completion of the Initial Decision.

[26] Interestingly, another group of investigators have suggested that:

> * * * Apparently water-restricted weanling mice lose their ability to regulate subsequent drinking behavior, and consume hyperosmola solutions whose osmolarity and sweetness would be aversive to humans.

(Takasaki, et al., Searle's Reply, Vol. 161, Appendix at 1).

carcinogenicity in which the agency has long exercised its considerable expertise (see, e.g., Commissioner's Decision: Cyclamate, 45 FR 61474, Sept. 16, 1980; Commissioner's Decision: FD&C Red No. 2, 45 FR 6253, Jan. 25, 1980; Commissioner's Decision: Diethylstilbestrol (DES), 44 FR 54852, Sept. 21, 1979). To assess the carcinogenic potential of food additives, the Bureau of Foods requires chronic studies in two rodent species, usually the rat and the mouse (Tr./I/page 16, lines 23–25). Proof of safety must entail negative findings from both species because it is not known which specie is more similar to man. As noted in Section IV(C) above, it is the agency's practice, in the absence of more precise scientific knowledge, to adopt findings from the most sensative species in order to maximize protection of the public health.

In keeping with the Bureau's requirements, Searle submitted chronic feeding studies on aspartame for both the rat (E–33/34, Vols. 43–44; E–70, Vol.81) and the mouse (E–75, Vol. 82). Similar studies were performed on the breakdown product diketopiperazine (DKP), also in both species (E–77/78 in the rat, Vols. 89–90, and E–76 in the mouse, Vol. 88). Because the parties have agreed that the mouse data are negative, only the three rat studies were subject to the Board's review and are considered in this decision.

These three rat studies had the following designs:

a. *E–33/34* was a 104-week study on aspartame, with exposure beginning after weaning. Dose levels were 0, 1, 2, 4, and 6–8 g/dg body weight.

b. *E–70* was a two generation study with aspartame exposure *in utero,* during lactation, and then for 104 weeks. Dose levels were 0, 2 and 4 g/kg body weight.

c. *E–77/78* was a 115-week study on DKP, with exposure beginning after weaning, as in E–33/34. Dose levels used were 0, 0.75, 1.5 and 3.0 g/kg body weight.

In all three studies, the test animal used was the Charles River CD (Sprague-Dawley) albino rat.[27]

The agency has set forth the general principles of statistical and biological significance which guide the evaluation of carcinogenicity studies (45 FR at 61477–81). Based on these criteria, studies may be classified as: (a) Positive; (b) inconclusive but suggestive of a carcinogenic effect ("suggestive"); (c) negative; or (d) deficient (*id.* at 61481, col. 2). In the case of aspartame, the parties dispute the proper category into which the three rat studies should be placed.

3. *Positions of the parties.* Dr. Olney would classify E–33/34 as suggestive and E–70 as deficient, thereby concluding that Searle's petition should not be approved without further testing. The central thesis in Dr. Olney's position is that the spontaneous rate of brain tumors in Sprague-Dawley rats, as reported in the scientific literature, is significantly below the incidence of brain tumors found in both the *control* animals in E–70 and the *treated* animals in E–33/34. Dr. Olney also considers the data in E–33/34, on their own, to suggest a dose response and accelerated tumor onset, both indicators of possible carcinogencity.

The Bureau of Foods and Searle consider both aspartame rat studies to be negative, thereby justifying approval of the food additive petition. In response to Dr. Olney's concerns, they maintain that the incidence rates at issue in the aspartame studies represent normal levels of background spontaneous incidence, and that E–33/34 demonstrates neither a dose response nor early tumor onset.

4. *The Board's decision.* The Board agreed essentially with Dr. Olany that the background rate for spontaneous brain tumors in this strain of rat was very low, the Board finding the rate to be approximately 0.7%[28] (Board's Decision at 43–45). Given that determination, the Board dismissed the E–70 study as "bizarre" because the *control* group there showed a 3.5% incidence of brain tumors (*id.* at 47). Also based on its assessment of the background rate, the Board found that, regarding study E–33/34: "By itself, the 3.5% incidence of brain tumors (in the *treated* animals) gives cause for concern" (*id.* at 46). The Board's concern about E–33/34 was augmented by its agreement with Dr. Olney that the data suggested a dose response and that there was a high incidence of gliomas (primary brain tumors) at a relatively early age. Accordingly, the Board, like Dr. Olney, would also classify E–33/34 as a suggestive study and E–70 as a deficient one.

5. *Additional evidence.* After the Board issued its decision, Searley, as part of its exceptions, submitted a recently completed long-term study conducted on Wistar rats by the Japanese firm, Ajinomoto Co., Inc. (the Japanese study) (Searle's Exceptions, Appendix 2). The study tested aspartame as well as an aspartame-DKP mixture. Searle also submitted additional data on the spontaneous rate issue (Searle's Exceptions, Appendices 3 and 4), as did the Bureau of Foods (Bureau's Exceptions, Appendix 3).

Because this proceeding is intended to be a scientific inquiry aimed at evaluating the safety of aspartame using all the available evidence, I have considered these materials as evidence in this proceeding, acknowledging that neither the Board nor the participants to the hearing have commented on them. In so doing, I note that none of these additional materials have served as a central basis for my decision, but rather only confirm the large body of evidence presented at the hearing.

6. *Commissioner's decision:* With due respect to the Board, I disagree with its assessment of the background rate of spontaneous brain tumors in Charles River CD (Sprague-Dawley) rats, and, therefore, I also disagree with the Board's characterizations of studies E–70 and E–33/34, which characterizations, especially regarding E–70, were largely dependent on the background rate assessment. As is explained in more detail below, I agree with the Bureau of Foods that 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. Using this approach, I find that the data in E–33/34 do not suggest, in terms of biological significance, a dose-response relationship or early tumor onset. Accordingly, I conclude that the two aspartame studies reviewed by the Board are neither "bizarre" (E–70) nor even of major "concern" (E–33/34), but rather they are negative studies.

7 *Conduct of the studies.* Dr. Olney and Mr. Turner have questioned the manner in which the aspartame/DKP studies were conducted and their credibility and usefulness for meaningful interpretation. The Board considered these issues to be beyond the scope of its charge and declined to rule on them (Board's Decision at 6–8). Mr. Turner has taken exception to this decision by the Board and has requested that the Board be reconvened to consider these issues (Turner's Exceptions in their entirety). The conduct of the studies and Mr. Turner's request for a new hearing are discussed in detail in Section VI below.

[27] Sprague-Dawley is the general strain of rat used. Different commercial suppliers have developed their own colonies of Sprague-Dawley rats, and the Charles River Laboratories from Wilmington, Massachusetts is one such supplier. "CD" simply means caesarian-derived.

[28] The Board was not as conservative in its estimate as was Dr. Olney, who considered the background rate to be 0.15% (Tr./III/page 139, lines 6–23).

B. Background Rate for Spontaneous Brain Tumors in Charles River CD (Sprague-Dawley) Rats

1. *Overview.* As noted above, the cornerstone of the Board's decision is that the background rate for spontaneous tumors in Charles River CD (Sprague-Dawley) rats is very low, approximately 0.7% (Board's Decision at 43–45). Specifically, the Board cited on four studies which showed spontaneous brain tumor incidence rates of 0.09%, 0.6%, 0.7%, and 3.2%. The Board gave extra weight to the two studies showing 0.6% and 0.7% incidence rates because the rats in those studies were obtained from the same commercial source (Charles River Laboratories) as those used in the Searle studies. The Board gave less weight to the study showing a 3.2% incidence rate because the number of rats used in that study (125) was considered to be too small for a reliable determination of spontaneous tumor incidence (*id.*).

Both Searle and the Bureau of Foods have taken strong exception to this portion of the Board's opinion (Searle's Exceptions at 16–22 and Bureau's Exceptions at 24–32). In general, both Searle and the Bureau argue that the Board gave too much weight to the studies at the lower end of the spectrum and ignored additional studies in the administrative record which reported spontaneous incidence rates as high as 5%. Searle and the Bureau therefore conclude that the incidence rates reported in the Searle studies fall within the normal spontaneous range.

After a thorough review of the studies in the administrative record submitted on this subject, I conclude as follows: (1) No single study and no group of studies submitted in this proceeding are sufficient to stand alone as a definitive statement of the background rate for spontaneous brain tumors in this strain of rat; (2) although several studies cited by the Board do report spontaneous incidences in the area of 1% or lower, these studies are partially flawed and must be supplemented by other data presented at the hearing which reported incidence rates comparable to those in the Searle studies; [29] and, therefore: (3) the primary evaluation of the Searle studies should be on the basis of comparison with concurrent, not historical, control data.

Four factors seem to play a significant role in creating this spectrum of findings. The first is the variation that would be expected among tests run at different times and at different places by different people (Koestner Testimony, Tr./III/page 257, lines 21–25; see also MacKenzie and Garner, Vol. 134, Tab 20 at 1252–53).

A second factor is the size of the study population. The smaller the size of the test sample, the larger will be the variation associated with the estimated of the spontaneous incidence rate, and vice versa.

The third consideration is the methodology used, especially the meticulousness of the search. For example, studies in which animal organs are observed only by the naked eye are likely to turn up fewer tumors than would a study in which the animal organs are routinely examined under a microscope. Similarly, where more sections of the brain are examined, the chances are greater that tumors will be found, thereby increasing the tumor incidence reported (Tr./III/page 258, lines 1–9).

Finally, as the Board noted, the strain and commercial source of rat used are important because animals derived from different colonies may acquire different characteristics (Board's Decision at 45, citing MacKenzie and Garner, Vol. 134, Tab 20).

These four factors help explain why there are such varied results among the reported studies. As the Board noted, emphasizing different methodologies used:

> It is difficult to conclude from the archival literature which of various published figures most accurately reflects the "normal" (i.e., presumably non-toxogenic) incidence of brain tumors in the Sprague-Dawley rat strain. Several published reports are based on findings in rats that had been used in long-term studies designed to check the potential toxicity of a particular compound, or of irradiated foods. Other reports fail to state the protocol followed in examining the brain tumors: Gross-anatomical tumor identification only, or routine histological examination of each brain?

(Board's Decision at 43).[30] The published literature also varies considerably in terms of the study population size, the commercial source of rat used, and the time and place the data were collected.

These four factors also help explain why the Searle studies reported a wider range of incidence rates than reported in those studies relied on most heavily by the Board. First, the studies relied on by the Board were conducted at different laboratories than were Searle's studies. Indeed, the one background rate study in the record which was done by the same laboratory as were Searle's studies (at least in part) reported a higher incidence than did the studies relied on by the Board (Gart, et al, Vol. 154, Tab 7, Table 4, discussed below). Second, the test populations in the Searle studies tended to be smaller than in the studies relied on by the Board, thus increasing the variation observed in the spontaneous incidence rates. Third, in the Searle studies, a very detailed histopathological examination was performed (involving either 7 or 8 brain sections per animal) which increases the chances of detecting tumors. Finally, the Charles River rat used by Searle was not uniformly utilized in the reported literature relied upon by the Board (i.e., Mawdesley-Thomas and Newman study, discussed below).

Accordingly, I find that the spontaneous incidence rates in the Searle studies are consistent with the normal background rate, as determined from the data in the administrative record of this proceeding.

2. *Studies Relied Upon by the Board:* As noted above, the Board cited four studies in making its determination of the background rate for spontaneous brain tumors in Charles River CD (Sprague-Dawley) rats. These studies may be reviewed, as follows:

a. *Mawdesley-Thomas and Newman* (Vol. 135, Tab 18): This study reported 38 tumors (24 in males) in approximately 41,000 rats for an incidence of 0.09%. These rats were fed either a control diet on one of a variety of test compounds. The rats were of the general Sprague-Dawley strain but were not obtained from Charles River Laboratories.

Searle criticizes this study because, in a subsequent publication (Vol. 135, Tab 19), the same authors reported that the incidence rate in this study was probably closer to 1% than 0.1% (Searle's Exceptions at 16–17). The Bureau criticizes this study for three reasons: (1) Because the histological examination was more limited than in the Searle studies; (2) because tumors were eliminated whenever they were suspected of being compound-related; and (3) because not all the slides were reviewed by the authors themselves (Bureau's Exceptions at 26–27).

I agree with Searle, the Bureau, and the authors themselves that the reported incidence of 0.09% is probably too low. The test population included both treated and control animals. The authors eliminated any tumors from the incidence count that were "suspected" of being compound related, but did not also eliminate the other "treated" animals as well (Vol. 135, Tab 18 at 108). This approach likely inflated the number

[29] This conclusion is further supported by the spontaneous rate materials submitted with Searle's and the Bureau's exceptions.

[30] Indeed, one Bureau witness suggested that virtually all the reported studies, whether they reported high or low spontaneous rates, have some methodological deficiencies (Tr./III/page 195, lines 7–11).

of tumor-free animals and lowered the reported spontaneous incidence rate. It therefore appears that the authors' subsequent statement, that the true spontaneous incidence rate among this group of animals was probably higher than originally reported, is correct.

b. *MacKenzie and Garner* (Vol. 134, Tab 20): This study reported three brain tumors in a two-year study conducted on 535 rats, for an incidence rate of 0.6%. The test animals included both those fed irradiated feed and those on a control diet. Although a breakdown by sex is not given for these three tumors, when the authors combined all the strains of rats tested, over two-thirds of spontaneous brain tumors were found in males (Vol. 134, Tab 20 at 1251, col. 1).

Searle criticizes this study because the authors themselves (at page 1252 state that their findings cannot be compared with others' because of differences in methodology and diagnostic criteria (Searle's Exceptions at 17). The Bureau makes only the general criticism, as it does with the remaining two studies relied on by the Board, that there is not enough detail about the methodology to enable a correct assessment of the thoroughness of the histological examination (Bureau's Exceptions at 27).

Although this study is clearly entitled to some weight, I believe that the Board overemphasized its importance. The main purpose of this publication was to compare spontaneous incidence rates in studies conducted at different laboratories and at different times. And, indeed, the authors reported significant differences. Although it is true that the specific incidence rate relied upon by the Board was based on rats derived from the same commercial source as were the rats in the Searle studies, commercial source in only one of several factors that can affect the incidence rate (see discussion in this very study at 1252, col. 2). Moreover, the authors state that "many small tumors" found in other studies would not be called neoplasms by them (id.), a practice which could have lowered the reported incidence rate.

c. *Fitzgerald, et al.* (Vol. 134, Tab 22): This study reported five brain tumors in 650 rats for an incidence of 0.7%. Once again, some of the test animals were fed test compounds while others were on a control diet. The authors reported that these tumors predominated in the males, although an exact breakdown by sex was not given.

There are two weaknesses in this reported study. First, the authors did not state how many brain sections were routinely examined microscopically. As noted above, less-than-meticulous searches could have the effect of lowering the reported spontaneous incidence rate. Second, the authors reported that animals were sacrificed at unspecified intervals (Vol. 134, Tab 22 at 265). Early deaths may also have helped lower the reported incidence.

d. *Thompson, et al.* (Vol. 134, Tab 18): This study reported four brain tumors in 125 rats for an incidence of 3.2%. The Board found, however, that "[t]he number of rats used in this study is too small for a reliable determination of spontaneous-tumor incidence" (Board's Decision at 44). Searle believes the Thompson data are nevertheless valid, arguing that smaller study populations tend to produce a wider variation in reported incidence rates (Searle's Exceptions at 17–18).

Both the Board and Searle make valid points that are not mutually exclusive. As noted above, it is true that small test populations lead to greater imprecision in estimating spontaneous incidence rates. For this reason, the Thompson study would not serve as a reliable indicator of the "true" spontaneous rate. However, when a study's test population is small, the *variation* in the observed incidence rate will be large, and the frequency of observing both high and low incidence rates will be increased. Thus, due to this increased variability, the incidence in the relatively small Thompson study (4/125, 3.2%) is acceptable for that size test population. When the Thompson data are added to the other three studies cited by the Board (.09% [38/41,000], .6% [3/535], .7% [5/650] and 3.2% [4/125]), the spectrum is not unlike that in the three control groups in the Searle studies, .8% (1/119) in E–33/34, 1.6% (2/123) in E–77/78, and 3.5% (4/115) in E–70. Inclusion of the Thompson study in this type of comparison is valid because Thompson's study size (125) is comparable to those in the Searle control groups (approximately 120 each).

Dr. Olney suggests that the 3.2% figure is too high because three of the four tumors were found in animals fed a diet of irradiated feed (Olney's Exceptions at 2). Dr. Olney's point is valid to the extent that it indicates a flaw in this study (i.e., using both "treated" and "control" animals), but it is a flaw common to all of the studies relied upon by the Board. Such flaws underscore the need to consider the truly "control data" described below.

3. *Other evidence.* In addition to these four studies cited by the Board, I also consider the following data to be relevant:

a. *Gart, et al.* (Vol. 154, Tab 7, Table 4). These data were collected by the National Cancer Institute ("NCI") from *control* animals used in the carcinogenesis bioassay program. The participating organizations were NCI and Hazelton Laboratories, the laboratory used by Searle for studies E–33/34 and E–70 (Tr./III/page 214, lines 14–24 and page 217, lines 11–16). The data were derived from Charles River CD (Sprague-Dawley) rats and showed an incidence rate for brain tumors of 2.2% (8/368) (Tr./III/page 197, lines 13–16).

As Dr. Olney points out, it is true that these data are reported only in tabular form without a detailed description of the methodologies used (see Tr./III/page 218, lines 1–6). Nevertheless, the fact that these data were all derived from control animals is sufficient to consider the information in this proceeding, especially because there is such an overall sparceness of truly "control" incidence data available in the record (see Tr./III/page 217, lines 2–5).

The spontaneous incidence of brain tumors reported by NCI (2.2%) is approximately triple the incidence reported by the MacKenzie, et al. and Fitzgerald, et al. studies relied on heavily by the Board. Significantly, these data all were derived from the same commercial source (Charles River) and were housed, at least in part, in the same laboratory (Hazelton) used by Searle. As noted above, these factors are known to affect reported spontaneous incidence rates (MacKenzie and Garner, Vol. 134, Tab 20 at 1253, cols. 1–2).

One direct comparison between the NCI data and Searle's control data is quite striking. If the controls from all three Searle studies are combined, the resulting incidence rate is very comparable to the NCI data for sample populations of nearly identical size: 2.0% (7/356) for combined Searle control data and 2.2% (8/368) for NCI control data (Tr./III/page 195, line 22—page 196, line 9 and Tr./III/page 197, lines 13–16).[31]

b. *Additional Data.* Other relevant non-aspartame studies reported incidence rates for *control* animals of 5% (2/40), 3.3% (2/60) (reported twice), 2% (8/400), 1.9% (7/368), 1.5% (13/876), and 0.5% (3/575). Moreover, all of these data were based on control animals and were obtained from the Charles River Laboratories, the same source used by Searle. The utility of these data is somewhat limited because the data were not available for the Board's consideration (Searle's Exceptions,

[31] A second, more confirmatory type of comparison is that NCI and Searle both reported a higher spontaneous occurrence of brain tumors in males than in females. This is consistent with the other reported studies.

Appendices 3 and 4; Bureau's Exceptions, Appendence 3), and because they report findings only in summary form without any detailed description of the test methods.[32] Nevertheless, this information tends to confirm my conclusions drawn from the data presented at the hearing and therefore may be considered as additional support for those conclusions.

As noted above, several of these reported brain tumor incidences were significantly higher than those reported by the Board, with sizable studies reporting incidences of 1.5%, 1.9% and 2.0%. Moreover, one particular study illustrates quite well the point that small studies are subject to wider variation in reported spontaneous incidences (both higher and lower) than are larger studies. These data, which showed an overall spontaneous incidence rate for brain tumors of 2.0% (8/400) (Searle's Exceptions, Appendix 3) was actually broken down into the following four separate control groups that were run concurrently: 4% (4/100); 3% (3/100); 1% (1/100); and 0% (0/100). This variation is remarkably similar to that seen in the control data from the three Searle studies: 3.5% (4/115) in E-70; 1.6% (2/123) in E-77/78; and 0.8% (1/119) in E-33/34.

3. *Conclusions on spontaneous rate.* Based on the above analysis, I find that the Board's conclusion that the background rate for spontaneous brain tumors in Charles River CD (Sprague-Dawley) rats was approximately 0.7% was unduly low. Although it is true that three studies relied on by the Board showed tumor incidences of less than 1%, these studies each had some flaws, or discussed above, and other credible data reported spontaneous incidence rates for brain tumors in the mid-1% and 2% range. Also important are additional data derived from relatively small studies (comparable to E-70 controls) reporting spontaneous incidences in the 3% range or even higher, which results may be attributed to the variation that may reasonably be expected from studies with small sample populations. I therefore find the historical control data to be consistent with the rates reported in the Searle studies, and therefore the safety of aspartame should be evaluated on the basis of comparison with concurrent controls.

C. Studies on Aspartame and DKP

1. *General principles.* It is generally accepted that "the first and foremost comparison of a treated group is to its concurrent controls" (Gart, et al., Vol. 155, Tab 7 at 962). As noted above, the agency has set forth general principles of statistical and biological significance which guide the evaluation of carcinogenicity studies (45 FR 61477-81). Factors usually considered are: "the methodology of the study involved, the existence of a dose response relationship, the rarity of tumors, and the presence of similar results in other studies" (*id.* at 61478, col. 2). Other relevant considerations include the tumor incidence in treated animals versus concurrent controls (Tr./III/page 190, lines 13-17), any acceleration of tumor onset (Tr./III/page 191, lines 1-2; Tr./III/page 250, lines 14-15), and study size (45 FR at 61482).

These criteria will be discussed below in the context of the Searle studies in which they arise.

2. *E-33/34 (Vols. 43-44)* [33]—a. *Study Design.* This study was conducted on Charles River CD (Sprague-Dawley) rats using aspartame as the test compound. Four treatment groups, consisting of 40 rats per sex per group, were fed aspartame as part of their regular diets at dosage levels of 1, 2, 4, and 6-8 g/kg body weight/day, respectively, for a period of 2 years, beginning after weaning at 4 weeks of age. A control group of 60 rats per sex were fed the same diet without the aspartame. At the conclusion of the 2-year period, all the surviving test animals were sacrificed, and their brains (as well as other organs) were examined histologically. Eight coronal sections were eventually examined from each animal's brain.

b. *Study Results.* Examination of the brains revealed a total of 13 tumors, one in the control group and 12 spread among the four treatment groups (Board at 40-41). The breakdown by sex and dosage level is as follows:

Group	Males	Females
Control	1/59	0/59
1 g/kg	2/36	2/40
2 g/kg	1/40	0/40
4 g/kg	4/40	1/40
6-8 g/kg	0/39	2/38

The numerators shown above represent the figures found by the Board (*Id.*). The denominators represent the total number of animals at risk which were verified by UAREP (Vol. 111 at 391, Table IV-20].[34] Most of these tumors were gliomas, although one tumor in the high dose female group was diagnosed by the Board as a medulloblastoma (Board's Decision at 40-41).

Before analyzing the data, it is necessary to resolve two disputes about exactly how many tumors were found among the test animals. The controversy is due to variations in tumor count among the several persons or groups who viewed the slides: Dr. Innes on behalf of Searle,[35] the UAREP Committee, and the Board.

(1) *Male control group:* In this category, Dr. Innes reported no tumors, while UAREP and the Board each reported one tumor. Dr. Olney took exception to this finding by the Board, asserting that the Board's diagnosis, "most likely a metastatic carcinoma," meant that it was not a primary brain tumor (Olney's Exceptions at 2). The Bureau and Searle each counted this tumor, relying on UAREP's diagnosis that the tumor was an astrocytoma (Bureau's Exceptions at 14; Searle's Exceptions at 26). Searle also asserts that, in the absence of carcinomas found in other organs, the tumor could not have been metastatic [36] (Searle's Exceptions at 26).

I agree with the Board, UAREP, the Bureau and Searle that this tumor should be counted, for several reasons. First, tumor findings by a group with UAREP's expertise are entitled to considerable weight, especially positive findings, which are much more difficult to discount than negative ones. Second, although the Board "tentatively" diagnosed the tumor as metastatic (it used the qualifying words "most likely"), the Board did include this tumor in its control group count (0.8% vs. 0.0%), something the Board should not have done had the tumor been clearly metastatic. Finally, even by including this tumor, the control incidence is still only 0.8%, which lies at the lower end of the spontaneous incidence spectrum (see Subsection B above). Accordingly, the weight of the evidence strongly

[32] The one exception was the study reporting a 5% incidence (Ulland, et al., Vol. 155, Tab 4), to which neither shortcoming applies.

[33] This study is reported at several different places in the administrative record. The original report is designated as E-33/34 and is found in volumes 43 and 44. That report is supplemented by a pathology report performed for Searle, designated as E-87 or the "Innes Report," and found in volume 98. Both reports were subsequently reviewed by UAREP in Vol. 110, pages 5-14, Vol. 111, pages 256-457, and Vol. 112, pages 833-45, as part of the authentication procedure described in Section I above.

[34] Decreasing the denominators for this reason makes the data base more accurate and reliable. No appreciable effect is seen in the statistical evaluations as a result of this change. Statistical results reported in this decision are based on the data base listed above and therefore vary slightly from the results reported by the Bureau of Foods.

[35] Dr. Innes' review superseded an earlier review conducted by Experimental Pathology Laboratories (EPL), also on behalf of Searle. Dr. Innes' review was based on a more detailed sectioning of the test animals' brains than was EPL's review (see UAREP Report, Vol. 112 at 833-45).

[36] The term "metastatic" means that the tumor originated at another site and then transferred to the brain.

favors treating this tumor as a primary brain tumor.

(2) *1 g/kg male group:* In this category, Dr. Innes and the Board reported two tumors while UAREP reported only one tumor. Because it is more likely that a qualified pathologist might miss one tumor than incorrectly diagnose a non-tumor as a tumor, I agree with Dr. Innes and the Board that two tumors should be counted in this group.

c. *Analysis*— (1) *Board's decision.* The Board considered this study to be suggestive of causing brain tumors, for three reasons: (1) The increased incidence of brain tumors in aspartame-fed rats (reported as 3.75%) when compared to historical controls; (2) a possible dose-response, as seen by comparing the incidences in the lower two treatment groups combined (3.1%) with that of the upper two treatment groups combined (4.3%); and (3) the prevalence of early-occurring gliomas, two allegedly in the first year of life and three in the second year (Board's Decision at 41 and 46–47).

(2) *Positions of the parties.* Both Searle and the Bureau filed extensive exceptions to this portion of the Board's decision (Searle's Exceptions at 22–29 and Bureau's Exceptions at 14–22 and 32–35). Principally, they claim: (1) Appropriate statistical analyses show no significant increase in tumor incidence in the treated animals when compared to concurrent controls; (2) the Board's method for evaluating a dose response was not valid, and more appropriate tests show no dose response; and (3) the Board made factual errors in noting the time of death for certain rats.

(3) *Study evaluation.* In evaluating this study, data for the males and females have been analyzed separately. This is because the treated males lived longer than their concurrent control counterparts, and the treated females died sooner (Tr./III/page 323, line 19 to page 233, line 11). Moreover, the males produced more tumors than the females, which is consistent with the results in the background studies discussed in Subsection B above, especially, Gart, et al (Vol. 154, Tab 7 at Table 4).[37] Using this approach, study E–33/34 may be evaluated as follows:

[37] One acknowledged inconsistency with this Decision is that the background rate issue has been analyzed by combining the sexes while the comparisons to concurrent controls is being analyzed with males and females separately. Sexes were combined in the background rate analysis because the reported studies relied on by the Board did not give a breakdown by sex. However, the Searle studies do give such a breakdown, and for the reasons stated in the text, a separate anslysis for males and females is appropriate (*cf.* 45 FR at 61486, col. 2 and 61489, col. 2).

(a) *Tumor incidence.* The tumor incidences found, stated in percentage form, are as follows:

Group (grams per kilogram)	Males (percent)	Females (percent)
Control	1.7	0.0
1	5.8	5.0
2	2.5	0.0
4	10.0	2.5
6–8	0.0	5.3

Tumor incidence has been analyzed statistically by the Bureau of Foods using the Fishers Exact test, one-tailed (Tr./III/page 198, lines 21–22). This test calculates the probability of obtaining the observed or more extreme results, if there was no difference between the treated and control groups. The smaller the calculated probabilities, the greater the likelihood that the results are not due to chance alone, but may be treatment-related (see 45 FR at 61478, col. 1).

The P values from the Fishers Exact test are as follows:

Group (grams per kilogram)	Males	Females
1	0.32	0.16
2	0.65	1.00
4	0.08	0.40
6–8	1.00	0.15

Although at least one P value (P = .08, 4 g/kg males) may in some cases be cause for concern, it is not a cause for concern here because the finding is not repeated in any other dosage group because the males did not exhibit a dose response. As a general rule:

> The factors to be considered in determining biological significance [including lack of a dose response] may increase or *decrease* that confidence [that may otherwise be placed in low P values].

(45 FR at 61481, col. 1 (emphasis added); *cf.* 45 FR at 61478, col. 3). I therefore find that the tumor incidence analysis does not indicate biologically significant findings.

(b) *Dose response.* The Board concluded that the data suggest a dose-response relationship (Board's Decision at 46–47). The Board reached this conclusion by combining the data as follows, as advocated by Dr. Olney (Tr./III/page 126, lines 12–16):

Lower two groups combined	Upper two groups combined (percent)
Both sexes combined 3.1 percent	4.3

Although the Board did not separate the animals by sex, similar results are found if this is done:

Lower two groups combined (percent)	Upper two groups combined (percent)
Males 3.9	5.0
Females 2.5	3.8

Both the Bureau and Searle take exception to the Board's conclusion on dose-response, asserting: (1) That the dosage levels should not be combined in this fashion, and (2) the data do not produce biologically significant results using appropriate analyses (Searle's Exceptions at 24–26; Bureau's Exceptions at 21–22).

I find that the statistical trend tests utilized by the Bureau of Foods (Cox and Breslow tests), which simultaneously consider all dose levels, are more appropriate for analyzing these data than is the method used by Dr. Olney and the Board. These trend tests are especially useful for the data in E–33/34 because both tests account for differing survival times between the treated and control groups of each sex and make the appropriate adjustments. The Breslow test also gives extra weight to tumors which are observed early (Tr./III/page 200, line 23—page 202, line 3). The Cox and Breslow tests for trends yield the following P values:

	Males	Females
Cox	0.44	0.04
Breslow	0.47	0.02

The P values raising obvious concern are those for the females. The Bureau argues that these values are not biologically significant because they are largely dependent on a single medulloblastoma (found in one of the high dose females at 12 weeks) which, accordingly to the Bureau, was probably not caused by aspartame (Bureau's Exceptions at 32–35; see also Searle's Exceptions at 27–29). If the medulloblastoma is excluded from these analyses, the P values become 0.15 for the Cox test and 0.13 for the Breslow test.

The crux of the Bureau's argument is that, because the medulloblastoma caused death at age 12 weeks, the tumor most likely originated in embryonic brain tissue before aspartame was ever administrated. (In E–33/34, ingestion of aspartame began after weaning, at four weeks of age.) The Bureau believes its hypothesis is confirmed because of the failure to detect any additional medulloblastomas either in this study or in E–70, where the animals were exposed to aspartame *in utero,* during

lactation and then for 104 weeks. Searle agrees with the Bureau on these points.

Whenever a single tumor has such a large impact on observed probabilities, caution should be used in evaluating the results from any statistical test. Under the unique facts presented here, I agree with the Bureau of Foods that the results from the Cox and Breslow tests should not be considered biologically significant. This tumor likely originated in embryonic tissue before aspartame was administered, and the absence of additional medulloblastomas in this study or in E-70 support this conclusion (Tr./III/page 204, lines 2-19 and page 262, line 23—page 263, line 11). I therefore conclude that E-33/34 does not exhibit a dose response.

(c) *Time of tumor onset:* The Board was also concerned about what it perceived as a "high incidence of gliomas at a relatively early age: 5 rats died with glioma before completing the second year of life" (Board's Decision at 46). According to the Board, these animals died at weeks 8, 16, 66, 84 and 100 *(id.)*.

Both the Bureau and Searle claim that the Board made a factual error with respect to the two animals which allegedly died with a glioma at age 8 and 16 weeks. Searle and the Bureau assert that the "8 week" animal really died at 68 or 69 weeks, and the "16 week" animal really died at 76 weeks (Searle's Exceptions at 23 and Bureau's Exceptions at 15). Thus, the dispute here is whether these animals died early in their first year or well into their second year of life.

After a review of the relevant documents, I agree with Searle and the Bureau that the Board did indeed make two factual errors. In actuality, these animals died at approximately 69 and 76 weeks, respectively (UAREP, Vol. 111 at 403 [Animal No. 83-766] and 396 (Animal No. 83-837)).

The corrected figures are certainly less dramatic; all animals with gliomas died either during the second year of life or were sacrificed at the conclusion of the study. Moreover, none of the gliomas were confirmed as being the cause of death. As Dr. Koestner testified at the hearing:

> * * * these animals just happened to die from a non-tumor related cause and histological examination of the brain reveals an unexpected microtumor which eventually would have shown up as a grossly detectable neoplasm had the animal been permitted to live.

(Tr./III/page 255, lines 18-22; see also Tr./III/page 225, lines 5-11). Accordingly, I find that there are no biologically significant findings of early tumor onset.

(3) *Conclusion on E-33/34:* For the reasons stated above, I consider E-33/34 to be a negative study.

3. *Study E-70* (Vol. 80) [38]—a. *Study design.* This study was also conducted on Charles River CD (Sprague-Dawley) albino rats using aspartame as the test compound. The protocol differed from E-33/34 in that the treated animals were esposed to aspartame, through their mother's diets, both *in utero* and during lactation, and then for 104 weeks as part of their own diets. The Bureau requested Searle to perform a study with aspartame exposure beginning at conception because of the known sensitivity of the fetal or infant rodent to toxic effects from high doses of glutamic acid and aspartic acid (Tr./III/page 205, lines 18-24) (see generally Section IV(C) above).

E-70 used two dosage levels, 2 and 4 g/kg body weight/day, in groups of 40 animals per sex. A control group originally consisting of 60 animals per sex was also used. A treatment group comparable to the highest dose in the E-33/34 study (6-8 g/kg) could not be used because of exhibited non-specific toxic effects in fetal tissue caused by decreased food consumption in the mother (Tr./III/page 206, lines 11-24).

Test animals were necropsied at the time of death, or at 104 weeks after weaning, whichever occurred first. Eight brains sections per animal were examined histologically.

b. *Study results.* The tumor count [39] and the number of animals at risk (as verified by UAREP [Vol. 111, page 559, Table V-20]) are as follows:

Grams per kilogram	Males	Females
Controls	3/58	1/57
2	2/36	1/39
4	1/40	1/40

c. *Analysis*—(1) *The Board's Decision:* As noted above, the Board discounted completely the results from this study, calling them "bizarre" because the incidence of brain tumors in control animals, 3.5% (4/115) was considered completely out of line with the background rate in historical controls (Board's Decision at 47). The Board also found that the brain tumor incidence of the aspartame-treated groups combined, 2.5% (4/157), was "well above the normative figures" (*id.*). Finally, the Board criticized the study's size, stating: "this critically important study should have included a larger number of experimental animals" (*id.*).

(2) *Positions of the parties.* The Bureau and Searle have taken exception to this portion of Board's decision also (Searle's Exceptions at 29-30 and Bureau's Exceptions at 35-36). They argue that the incidence rates are consistent with a correct assessment of the historical control data, and that this study, evaluated on its own, showed neither statistically nor biologically significant findings. Dr. Olney agrees with the Board that this study is deficient, due to his comparison to the historical control data (Tr./III/page 154, lines 1-3).

(3) *Evaluation of study.* As explained in detail in Subsection B above, I disagree with the Board's determination of the background rate for spontaneous brain tumors in Charles River CD (Sprague-Dawley) rats and, accordingly, I disagree with the Board's dismissing the results of this study.

Based on comparisons with concurrent controls, it is clear that this is a negative study, As Dr. Olney admitted at the hearing:

> As one can see, there is no significant difference between the incidence of brain tumors between control and experimental animals in the second aspartame study.

(Tr./III/page 153, line 24-page 154, line 1). This conclusion was confirmed by statistical analyses performed by the Bureau of Foods (Tr./III/page 207, line 2-page 208, line 19). Because of the *in utero* exposure, the results of this study alleviate the concern raised by Dr. Olney of increased risk to children in terms of brain tumors (Koestner, Vol. 152, Section XI at 10).

One additional point which needs to be addressed briefly is study size. As noted above, the Board suggested that this study should have included more animals. The protocol used in E-70 called for 40 rats/sex in each of the two treated groups and 60 rats/sex in the control group. Searle has demonstrated that this allocation of treated and control animals is comparable to the Bureau's *current* allocation standard (50 animals/sex for both treated and control groups) in terms of its ability to detect an increased tumor rate (Searle's Exceptions at 40, Chart 1). Thus, I do not share the Board's concern about study size.

[38] Like E-33/34, this study is reported in its original form (Vol. 80), in a pathology report by Dr. Innes (E-87, Vol. 98), and in review form by UAREP (Vol. 110 at 5-15; Vol. 111 at 458-577; and Vol. 112 at 833-45).

[39] Although the Board reported only two tumors in the 2 g/kg group (both sexes combined (Board's Decision at 42)), Dr. Innes and UAREP each reported three tumors, two in the males and one in the females (Vol. 112 at 838, Table 9-1). The omission of the third tumor may have been an oversight by the Board. All three have been counted here.

(d) *Conclusion on E-70.* For the reasons stated above, I consider E-70 to be a negative study.

4. *E-77/78* (Vols. 89-90) [40]—a. *Study Design.* This study differed from E-33/34 and E-70 in that the test compound used was diketopiperazine (DKP), a breakdown product of aspartame (less than 2%) (Vol. 112 at 30-31). Charles River CD (Sprague-Dawley) albino rats were also used in this study. Three treatment groups, consisting of 36 rats per sex per group, were fed DKP as part of their regular diets at dosage levels of 0.75, 1.5 and 3.0 g/kg body weight/day for 115 weeks, beginning after weaning. A control group of 72 animals per sex was fed the same diet without DKP. Test animals were sacrificed at the end of the dosing period, and their brains (seven sections per animal) were examined histologically.

b. *Study results.* The Board reported the following results and offered the following evaluation:

> In the E-77/78 study concerning the diketopiperazine of aspartame 5 tumors were recorded: 2 in the control group of 123 rats (1.6%), and the remaining 3 among the 198 animals of the three experimental groups (1.5%). Two of the 5 gliomas could have been noted on gross inspection of the brain.
>
> This study shows no difference between experimental and control groups, and the recorded percentages fall within the high range of normal incidence reported from various normative studies.

(Board's Decision at 43)

c. *Analysis of and Conclusion on E-77/78.* None of the hearing participants challenge this interpretation of the data. Accordingly, I agree with the Board that E-77/78 is a negative study.

5. *Additional evidence: The Japanese study* (Searle's Exceptions, Appendix 2). This study was conducted only recently by the Japanese firm Ajinomoto Co., Inc., and concluded after the Board issued its decision. A preliminary report was submitted by Searle as part of its exceptions. I have considered this study as evidence in this proceeding, acknowledging that neither the Board nor the hearing participants have formally commented on it.

The preliminary report at (page 1) contains the following summary:

> The brain tumorgenicity of aspartame (APM) and of its diketopiperazine (DKP) was studied in 860 SLC Wistar rats. APM at dietary levels of 1 g/kg, 2 g/kg, 4 g/kg or AMP+DKP (3:1) 4 g/kg was fed for 104 weeks. One atypical astrocytoma was found in a control rat and 2 astrocytomas, 2 oligodendrogliomas and 1 ependymoma were scattered among the 4 test groups. There was no significant difference in the incidence of brain tumors between control and test groups. It is concluded that neither APM nor DKP caused brain tumors in rats in this study.

Taking the available information at face value, this appears to be a negative study in terms of brain tumors. Without a review of the Bureau of Foods, however, as well as by other interested parties, I do not believe it proper to base approval of aspartame on this study's results. Nor is such a course necessary in this instance. The three chronic studies discussed above (E-33/34, E-70, and E-77/78) are sufficient for me to make a final determination on the safety of aspartame in terms of its potential for brain tumors in rats. However, because the Japanese study suggests that aspartame does not cause brain tumors in a second strain of rat, the SLC Wistar rat, this study provides additional support for my conclusion on the brain tumor issue.

D. Conclusion on Brain Tumor Issue

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. Additional support for this conclusion is found in the Japanese study, 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.

VI. Mr. Turner's Appeal

Mr. Turner and Dr. Olney have repeatedly challenged the quality of data produced in Searle's animal studies. Indeed, Mr. Turner has petitioned for the Public Board of Inquiry to be reconvened because of the Board's refusal to consider what he called the "scientific validity" of the studies [41] (Vol. 153, Tab 187; see also Turner's Exceptions).

The Board disagreed with Mr. Turner's characterization that it failed to consider the "scientific validity" of the studies, asserting that the Board "did not exclude evidence relating to the quality or appropriateness of the experimental design of the studies or the scientific conclusions that can validly be drawn from the studies" (Board's Decision at 7). What the Board did decline to do was to "undertake a retrospective quality inspection of all the studies presented to it" which the Board considered had already been accomplished by UAREP and FDA (*id.*). Quite clearly, the Board considered its charge, as delineated in the June 1, 1979 Federal Register statement, to relate only to interpretation of the data and not conduct of the studies.

Both Searle and the Bureau agreed with the Board's ruling on Mr. Turner's appeal (Searle's Reply to Turner's Appeal, Vol. 157, Tab 200 and Searle's Reply to Turner's Exceptions; Bureau's Reply to Turner's Appeal, Vol. 157, Tab 208; and Bureau's Reply to Turner's Exceptions).

I believe the problem is partly one of semantics, as the phrase "scientific validity" may have several different meanings. The Board understood Mr. Turner to mean that it should redo UAREP's work which was to authenticate the data (i.e., make sure that the studies were actually conducted). Clearly, the board was correct in not attempting to repeat UAREP's work. The Board, in turn, uses the term "scientific validity" to mean the conclusions that can be drawn from the data presented, including study design. These conclusions were clearly within the Board's domain, and it was based on these considerations that the Board reached its ultimate findings. There is a third area, however, that lies somewhere between those two. This relates to the *manner* in which the studies were conducted. Even if the studies were not fraudulent, that does not necessarily mean that they were well conducted. A non-fraudulent study might be conducted in such a poor manner that its results would not be considered meaningful (*cf.* 45 FR at 61478, col. 2). As then FDA Chief Counsel Richard A. Merrill wrote to Mr. Turner on February 24, 1977, questions regarding the "execution of the studies" could be raised at the public hearing (Attachment No. 1 to Turner's Appeal, Vol. 153, Tab 187).

I conclude, however, that a new hearing need not be held. With one exception discussed below, Mr. Turner has not stated with particularity any deficiencies in the conduct of any of the pertinent studies which he believes, either alone or collectively, are sufficiently serious as to warrant a

[40] This study was not reviewed by Dr. Innes. Neither was it reviewed by UAREP, although a similar authentication was performed by the agency (vol. 151, Tab 167).

[41] Mr. Turner's full prayer for relief included:

1. An order directing the Board to reconvene and consider whether certain studies have been validated;

2. An order directing an additional Board or other public investigatory body to validate these studies; and

3. Withholding of aspartame's approval until such validation is completed.

(Vol. 153, Tab 187 at 24-25)

study's invalidation.[42] Rather, Mr. Turner's (and Dr. Olney's) main criticisms appear to be mere speculations which fail to raise any genuine issue of material fact.

For example, Mr. Turner and Dr. Olney rely heavily on the 1976 Congressional testimony of then Commissioner Alexander M. Schmidt who characterized Searle's animal laboratory practices as "sloppy" (Tr./III/page 129, lines 1–4). That testimony was based on findings of an FDA investigation of two of Searle's *drug* studies which only peripherally concerned aspartame. The relevance of this investigation to the aspartame proceeding is that it triggered the detailed audit conducted by UAREP and the agency, and therefore, for the purposes of this proceeding the drug study investigation was superceded by the UAREP/FDA audit. Nevertheless, based on the "sloppy" laboratory practices theory, Dr. Olney attributed the slightly higher incidence of brain tumors found in the E–70 control animals over concurrently treated animals to a hypothetical mix-up that may have occurred between the control and treated groups (Olney's Pre-Hearing Position Paper, Vol. 151, Tab 160, Part III at 15). The speculation inherent in this allegation was evidenced at the hearing when, as the issue of the higher control incidence in the E–70 animals arose, the following exchange took place:

> Dr. Spitznagel [Consultant to Dr. Olney]: Our only comment on that is we have our suspicions, mainly that some of the controls were actually treated.
>
> Dr. Bussey [Consultant to Searle]: Do you have evidence to that effect?
>
> Dr. Spitznagel: No, we really don't other than the assertion of the Commissioner of FDA.
>
> (Tr./III/page 242, lines 20–25).

The only specific allegation by either Mr. Turner or Dr. Olney relates to the E–77/78 carcinogenicity study conducted on DKP Dr. Olney cites a Bureau of Foods report that raises the possibility that the DKP-containing feed may not have been homogeneous (Report from Bureau of Foods' Task Force, September 29, 1979, pages 10–11, Volume 151, Tab 167). Dr. Olney's point here is that the non-homogeneous feed may have resulted in the "treated" animals' selectively not eating the DKP

The Bureau of Foods' documents at issue relate to the authentication review conducted by FDA.[43] The pertinent documents were placed into the record by the Bureau shortly after the hearing, at the request of Dr. Olney and Mr. Turner (Volume 151, Tab 167). The documents include portions of FDA's on/site inspection report of Searle as well as a Task Force memorandum interpreting and commenting on that report.

The agency's investigation culminated in a Bureau Task Force Report which thoroughly discussed the homogeneity issue. The Task Force concluded that, although the homogeneity issue could not be conclusively resolved, no serious problems were encountered which would invalidate the study. The remedy advocated by the Bureau, and adopted by the agency, was to notify Searle by letter of laboratory practices which should be corrected in the future (see Memorandum for the Files, dated September 26, 1977 prepared by Taylor M. Quinn, and draft letter to Searle from Commissioner Kennedy (undated), both in Vol. 151, Tab 167).

Dr. Olney's one piece of "hard evidence" was a photograph of a feed mixture showing DKP particles larger than that of the feed, so that the animals in the treated group might have discriminated in favor of the smaller non-DKP particles (photograph attached to Olney letter of February 6, 1980, Vol, 151, Tab 165).

I agree with the Bureau that the evidence is not sufficient to invalidate this study. The photograph in question was taken by a sample prepared especially for stability testing purposes, not feeding purposes. As the Task Force wrote: "it could not be determined whether these samples were representative of the diets fed to the rats, since the batches were made up specifically for this analysis and were made in smaller amounts" (Vol. 151, Tab 167, Task Force Report, Appendix A at 10–11). Thus, Dr. Olney's allegation here also appears to be speculative.

Nor is it necessary to order a new validation of these studies, as Mr. Turner suggests. Although the UAREP audit was undertaken to determine whether the aspartame studies were authentic or fraudulent, the three volume report covering over 1,000 pages contain detailed observations of how these studies were conducted.

UAREP has addressed itself to the question of whether the experiments were *carried out according to protocol plans* and the accuracy and reliability with which the experiments were performed and reported to the FDA. (Vol. 110 at 2) (emphasis added). Indeed, UAREP addressed such issues as: (1) Protocols; (2) clinical observations; (3) body weight, food, and compound consumption; (4) survival data; (5) clinical laboratory studies; (6) ophthalmoscopic observations; (7) necropsy; and (8) histopathology (Vol. 110 at 5–15) as well as (9) personnel, facilities and methods; (10) animals and animal care; and (11) data production, handling and storage (Vol. 110 at 20–22). The FDA portion of the audit had a similar scope. These are very similar subject areas to those which Mr. Turner raises in his appeal (see Vol. 153, Tab 187 at 14–15). Yet, not once does Mr. Turner cite examples from the UAREP report as evidence of poor conduct of the studies. His request for a new "validation" review, therefore, appears to be merely a fishing expedition for evidence of "sloppy" laboratory practices.

It should be emphasized that UAREP, a consortium of nine universities, has unquestioned expertise in the area of preclinical animal testing and that its review of Searle's studies was undertaken with complete neutrality. Although UAREP, like the agency, noted some procedures and irregularities that warrant improvement, none were of such a serious nature as to invalidate an entire study. Indeed, UAREP noted, and I agree, that review of the histopathologic slides provides a better basis for validation of the data than many of the other parameters (Vol. 110 at 23). On this point UAREP noted general agreement between its pathologists' reviews and the original diagnoses (*id.* at 24–25). UAREP also noted that both Searle and Hazelton Laboratories were accredited by the American Association for Accreditation of Laboratory Animal Care which, at the time, carried out the most through and critical nationwide evaluation of animal care facilities (*id.* at 20).

Therefore, based on the extensive information available in the record regarding the conduct of Searle's studies, and Mr. Turner's failure to raise with particularity any specific issues other than the one discussed above, Mr. Turner's appeal is denied.

VII. Conditions of Use

The third issue at the hearing was defined as follows:

> Based on answers to the above questions,
>
> (a) Should aspartame be allowed for use in foods, or, instead should approval of aspartame be withdrawn?
>
> (b) If aspartame is allowed for use in foods, i.e., if its approval is not withdrawn, what

[42] Mr. Turner has had ample opportunity to do so, either at the hearing, as part of his "appeal" submitted after the hearing, or as part of his exceptions filed after the Board's decision.

[43] E–77/78 was one of the three studies which FDA, rather than UAREP, audited (see Section I above).

conditions of use and labeling and label statements should be required, if any? (44 FR at 31717).

The conclusions reached in Sections IV and V above compel the conclusion that aspartame should be approved for use in certain foods, as listed in 21 CFR 172.804. Equally clear is the fact that the post-marketing restrictions advocated by Dr. Olney (restrict aspartame to use only by obese and diabetic patients) and Mr. Turner (require a warning statement on all labels stating that aspartame should not be used by children) are not supported by the scientific evidence.

The conditions for use stated in the aspartame regulations (21 CFR 172.804), including labeling requirements, are affirmed in their entirety. These labeling requirements include: (1) A prominently displayed alert to persons with PKU that the product contains phenylalanine ("Phenylketonurics: Contains Phenylalanine"); (2) directions not to use aspartame in cooking or baking because the compound loses its sweetness when exposed to prolonged heat; and (3) labeling in compliance with FDA's special dietary foods regulations (21 CFR Part 105) if the food containing aspartame purports, or is represented, to be for special dietary uses.

The safety evaluation in Section IV above calls for one additional post-marketing requirement. One assumption in this proceeding is that extremely high amounts of aspartame's component amino acids may cause brain damage. Aspartame is being approved only because the available data establish that the maximum projected consumption of aspartame is still far, far below any level even suspected of being toxic. Neverthless, prudence dictates that these estimated use levels be compared to actual use levels to ensure the validity of the safety assessment. As a condition for approval, therefore, Searle is to monitor the actual use levels of aspartame and to provide such information on aspartame's use to the Bureau of Foods as the Bureau may deem necessary by an order, in the form of a letter, to Searle.

VIII. Conclusions

Based on the foregoing, I conclude that:

1. Section 409(c)(3)(A) of the act (21 U.S.C. 348(c)(3)(A)) permits FDA to approve a food additive petition only if a fair evaluation of the data establishes that the food additive will be safe under its proposed uses. See Section II.

2. "Safe" means a reasonable certainty in the minds of competent scientists that the food additive will not be harmful under its proposed uses. See Section II.

3. The act places the burden on proving safety on the company seeking approval of the food additive petition. See Section II.

4. For Searle to obtain approval of its food additive petition, it must prove that the data in the record establish that there is a reasonable certainty that the proposed uses of aspartame will not be harmful. See Section II.

5. The data in the record establish that the maximum projected daily consumption of aspartame is 34 mg/kg/day. See Section IV(A).

6. Based on the maximum projected daily consumption, the data in the record establish that there is a reasonable certainty that the ingestion of aspartame, either alone or together with glutamate, does not pose a risk of contributing to mental retardation, brain damage, or undesirable effects on the neuroendocrine regulatory systems. See Sections IV (B) and (C).

7 The data in the record establish that there is a reasonable certainty that the ingestion of aspartame does not induce brain neoplasms (tumors) in the rat. See Section V.

8. Searle has met its burden of proving that aspartame is safe for its proposed uses. Aspartame should therefore be allowed for use in foods as set forth in 21 CFR 172.804. See Sections III, IV, and V.

9. All the conditions of use contained in the aspartame regulation (21 CFR 172.804), including labeling requirements, should be required. In addition, post-marketing surveillance by Searle of aspartame's actual use levels is necessary to ensure that actual use remains well below suspected toxic levels. See Section VII.

The foregoing Final Decision in its entirety constitutes my findings of fact and conclusions of law.

IX. Order

In accordance with subsections (c)(3)(A), (f)(1), and (f)(2) of section 409 of the act (21 U.S.C. 348(c)(3)(A), (f)(1), and (f)(2)) and 21 CFR 12.130, and under the authority delegated to the Commissioner (21 CFR 5.10 (formerly 21 CFR 5.1)), it is hereby ordered that:

1. Approval of the food additive petition for aspartame (FAP 3A2885) is granted.

2. The stay of the effectiveness of the regulation for aspartame (21 CFR 172.804) is vacated and the regulation reinstated.

3. As a further condition for approval not listed in 21 CFR 172.804, Searle is to monitor the actual use levels of aspartame and to provide such information on aspartame's use to the Bureau of Foods as the Bureau may by order deem necessary.

The Initial Decision of the Public Board of Inquiry is affirmed in part and reversed in part, as modified and supplemented herein.

In accordance with section 409(f)(3) of the act (21 U.S.C. 348(f)(3)), the effective date of this order is October 22, 1981.

Dated: July 18, 1981.

Arthur Hull Hayes, Jr.,

Commissioner of Food and Drugs.

Appendix A—Board's Decision on Potential Brain Damage From Phenylalanine

A. Diffuse Brain Damage Associated With Abnormally High Plasma-Phenylalanine Levels: Phenylketonuria

Phenylketonuria (PKU) is an inherited disorder in the metabolism of phenylalanine. It is transmitted by an autosomal recessive gene, and its incidence in the United States is about 1 in 15,000. The disorder results from the absence of an enzyme (phenylalanine hydroxylase) that converts phenylalanine (PHE) to tyrosine; as a consequence PHE accumulates in body tissues—including blood—in abnormally high concentration: in untreated phenylketonurics plasma-PHE levels usually range between 120–600 μmol/dl (20–100 mg %) instead of the normal 6–12 μmol/dl. Through mechanisms not yet fully understood, these grossly elevated PHE concentrations are correlated with severely impaired development of the immature brain in general, and of the myelin sheaths of its nerve fibers in particular. The clinical consequence of this developmental impairment is a profound mental retardation, often accompanied by epileptic seizures and chronic dermatitis. Children born with the enzyme deficiency can develop to adults of normal intelligence, provided their condition is recognized soon after birth, and appropriate dietary treatment instituted promptly thereafter. It is estimated that the PKU newborn loses one percentage point of future intellectual capacity for each postnatal week the condition goes unrecognized (cf. Dr. Richard Koch's testimony at the public hearing). Treatment is aimed at keeping plasma-PHE concentrations at or below 70–80 μmol/dl by restricting the dietary intake of PHE. If this preventative regimen is to successfully maintained, families with a phenylketonuric child must impose upon the child a strict dietary discipline that cannot be relaxed until the child is adolescent. It is important to note, however, that phenylketonuric mental

retardation is conditional upon *sustained* high plasma levels of PHE, in contrast to the more focal brain damage that can result—as will be emphasized in a subsequent section—from a single, short-lived surge of glutamic or aspartic acid concentration in the blood plasma.

The essential question with which the Board found itself confronted in examining the phenylalanine issue is: at what level of ingestion could aspartame induce a rise in plasma-PHE concentration to 100 μmol/dl or higher—the levels associated with impaired brain development? It is clear that this question is of particular importance in the case of children under 12, whose brain is still immature, and in the case of women in the child-bearing age. The importance of the question for the latter category is accentuated by the well-established fact that the placenta maintains between the maternal and fetal circulations a 1:2 gradient in the plasma concentrations of most amino acids, including phenylalanine. This means that or the fetal plasma-PHE concentration to reach the 100 μmol/dl level, the maternal plasma-PHE concentrations needs to rise no higher than 50 μmol/dl.

Of the evidence presented the Board considers the following data of particular significance:

1. In *normal human adults,* the ingestion of a single loading dose of 34 mg/kg body weight aspartame (the 99th percentile of projected aspartame consumption *for an entire day*) dissolved in orange juice induces a rise in plasma-PHE concentration from a fasting level of 6 μmol/dl to 11 μmol/dl, a level normally found in adults and children following ingestion of a protein-rich meal. This peak value is reached about one hour after the aspartame ingestion, and recedes to fasting level within about 8 hours.

Ingestion of larger loading doses induces proportionately higher plasma-PHE elevations. A 50 mg/kg loading dose (in a 60 kg person 3,000 mg aspartame, or 150 aspartame tablets, or 6 liters of aspartame-sweetened beverage, but with its 50% content of PHE equivalent to less than half the 4,000 mg PHE contained in one 4-oz. hamburger) causes the plasma-PHE level to rise from 6 to 16 μmol/dl. Following a 100 mg/kg loading dose (equivalent to 12 liters of aspartame-sweetened beverage consumed in a single sitting) the plasma-PHE level rises to 20 μmol/dl. Only a 200 mg/kg loading dose was found to induce a rise to 50 μmol/dl, and only following this very large dose did the plasma-PHE concentration take more than 8 hours to return to baseline. This 200 mg/kg dose corresponds to 600 aspartame tablets, or 24 liters of aspartame-sweetened beverage consumed in a single sitting by a 60-kg adult, or to 100 tablets of 20 mg aspartame accidentally ingested by a 3-year old child. Only in this grossly abusive amount could aspartame ingested by a pregnant woman be expected to induce plasma-PHE concentrations high enough to cause, through placental transfer, fetal plasma-PHE levels approaching—for a few hours at least—the lower limit of potential toxicity. However, it seems inconceivable that so large a dose would be taken in a single sitting. When consumed over a 16-hour period—as would seem nearly unavoidable—it would undoubtedly induce a more sustained plasma-PHE elevation remaining well below the 50 μmol/dl peak induced by the same amount of aspartame taken as a loading dose.

2. In the normal *one-year old infant,* a loading dose of 34 mg/kg body weight causes the plasma-PHE concentration to rise from a fasting level of 6 μmol/dl to 10 μmol/dl, receding to baseline within 4 hours. It appears from this finding that the 1-year old normal child metabolizes PHE at least as effectively as does the normal adult.

3. In *individuals heterozygous for phenylketonuria,* a 34 mg/kg loading dose of aspartame induces a higher and longer-lasting plasma-PHE elevation. Instead of the 11 μmol/dl peak resulting from such a loading dose in the normal human, the peak reaches 16 μmol/dl in the PKU heterozygote and, in addition, the plasma-PHE curve declines more slowly than it does in normal individuals. A loading dose of 100 mg/kg aspartame—an abuse load even when ingested over a 16-hour period—is followed by a plasma-PHE rise reaching 42 μmol/dl, about twice as high as in the normal human. Even following this enormous single load, however, the peak value remains below the level at which, in the case of a pregnant woman, a risk to her unborn child might arise. Moreover, an abuse dose of 100 mg/kg aspartame would in the real-life situation not be ingested in a single sitting, as it was in the cited experiments, but, rather, consumed over an extended time period. Under these more natural conditions, the plasma-PHE concentration could be expected to remain well below the 42 μmol/dl level. It is of interest to note that a 100 mg/kg intake of aspartame by a 60-kg woman would add less to her dietary PHE consumption than would be added by an extra 4-oz. hamburger: 3,000 instead of 4,000 mg PHE.

4. *Undetected cases of phenylketonuria.* The question has been raised whether a risk might occur in unidentified PKU children as a consequence of the presence of aspartame in the food supply. The number of children in this category is unknown but thought to be very small. Screening of newborns for PKU is mandatory in 47 states, and it has been estimated that about 10% of the 200 PKU children born annually in the United States might remain undiagnosed and hence at great risk to grow up retarded (cf. Dr. Richard Koch's testimony at the public hearing). An undetected phenylketonuric infant would be adversely affected by the phenylalanine provided in breast milk protein (or infant formula) which may furnish levels of phenylalanine intake in the vicinity of 80 mg/kg/day. (This compares with a projected mean phenylalanine intake from aspartame in children under 2 years of 3 mg/kg/day). The argument that asartame in the food supply would significantly increase the risk of mental retardation in the unidentified phenylketonuric is not supported by these considerations. An undiagnosed PKU child is at risk first and foremost by being undiagnosed and hence permitted to consume meals that are standard for normal children. This point is emphasized further under the next item of consideration.

5. *PKU children who are not on a restricted diet.* As PKU children get older they may be allowed larger helpings of "free" food or they even go off their earlier retricted diet. This may not be harmful provided that the child's tolerance to phenylalanine is carefully monitored by blood tests. However, the question arises whether the availability of aspartame in the food supply would compromise the health and well-being of PKU children in this category. There appear to exist no explicit data based on controlled studies to answer this question, but it is possible to seek an answer by considering the amounts of phenylalanine that such children would be exposed to through usual food sources, in comparison with the PHE provided by aspartame. For example, a 4-oz. hamburger supplies about 4,000 mg phenylalanine, and a normal child would consume an average of about 200 mg phenylalanine per kg/day from normal food protein sources. This intake level compares with a projected daily aspartame-based phenylalanine intake of 17 mg/kg by those children whose aspartame consumption would reach the upper 99th percentile of the population. (For a 30-kg child this would correspond to a daily consumption of 2 helpings of

aspartame-coated breakfast cereal plus 8 cans of aspartame-sweetened beverage). Thus, for children on an unrestricted diet aspartame ingestion even at this high level would contribute less than 10% of the total daily PHE intake. For children whose protein intake is restricted the relationships between food protein-derived and aspartame-derived phenylalanine would differ, but again the total intake provided by aspartame remains small. In considering the daily variation in protein intake and the concentration of phenylalanine provided by normal foods it is evident that the ingestion of aspartame could not pose a significant extra risk to PKU children whose diet is either not restricted or only partially restricted. The significant risk to their health is clearly from the phenylalanine in the protein furnished by standard foods: In a 30 kg youngster one extra hamburger would add 100–150 mg/kg, one extra hot dog about 50 mg/kg, one extra glass of milk 15 mg/kg or nearly as much as the total amount of PHE supplied by a 34 mg/kg intake of aspartame.

6. *Hyperphenylalaninemia.* This term refers to a condition in which plasma-PHE levels anomalously range between 25 and 120 μmol/dl. Most of those afflicted with this abnormality are of normal intellect, and since they are usually asymptomatic also, neither they nor others are likely to be aware of their condition unless it has been identified by a newborn-screening test. The incidence of hyperphenlyalaninemia is about 1/30000, and it has been estimated that in the United States the condition affects about 1,750 women of childbearing age. It is this latter category that gives the most reason for concern, since the 50% among these women who have plasma-PHE levels ranging between 60 an 120 μmol/dl are at high risk of giving birth to brain-damaged children destined to grow up mentally retarded. The only effective prevention of this consequence of hyperphenylalaninemia would consist in a systematic reduction of dietary PHE intake through pregnancy—in other words, in treating the prospective mother much as a phenylketonuric child would be treated. Such prophylactic measures, however, are naturally contingent upon identification of the anomalous condition before or shortly after the beginning of the pregnancy. It follows that until such time as all hyperphenylalaninemics are identified by screening tests a complete prevention of congenital brain damage caused by maternal hyperphenylalaninemia cannot realistically be hoped for.

In evaluating the risk inherent in aspartame consumption by hyperphenylalaninemics, it is obvious that aspartame as a source of PHE can only contribute further to the already high plasma-PHE levels. It should be considered, however, that even the unlikely abuse intake of 100 mg/kg of aspartame per day by a 60-kg woman would supply less PHE (3,000 mg) than would be supplied by an extra 4-oz. hamburger (4,000 mg), and that the more likely (although still very high) intake of 34 mg/kg/day would be the PHE-equivalent of little more than two extra glasses of milk. It thus seems fair to conclude that the hyperphenylalaninemic woman is at much higher risk from the consumption of natural foods that she would be from the use of aspartame. It should be reiterated that the real problem of hyperphenylalaninemia lies in the usually covert nature of the anomaly.

Conclusions Regarding Aspartame-Induced Mental Retardation

In the Board's opinion, *aspartame consumption by normal humans* cannot be expected to increase the incidence of that particular form of mental retardation that is associated with sustained elevation of plasma-PHE levels to (or beyond) 120 μmol/dl during immature stages of brain development. This conclusion is based on the consideration that even the highly unlikely daily consumption level of 100 mg/kg of aspartame (3 times the projected upper one-percentile of aspartame consumption) would add no more than 15–20% to the normal dietary PHE intake, less than would be added in a 60–kg individual by an extra 4–oz. hamburger. Consumed at the estimated upper one-percentile level of 34 mg/kg/day, aspartame would increase the normal daily intake of PHE by no more than six percent. These figures lie well within the limits of day-to-day variations in dietary protein consumption.

In *individuals on a PHE-restricted diet* designed to prevent critically elevated plasma-PHE levels, aspartame is to be handled as any other source of phenylalanine. Since these individuals (phenylketonuric children and pregnant women known to have hyperphenylalaninamia) would follow a carefully prescribed diet, a cautionary label explicitly identifying aspartame as a PHE source should forestall a liberal use of this sweetener by such patients.

In the unfortunate case of *unidentified hyperphenylalaninemia,* the normal food-derived PHE poses a much greater risk to the patient (or the unborn child) than would aspartame, even when consumed in very large amounts. The hyperphenylalaninemic gravida not on a PHE-restricted diet would add 5–6% to her dietary PHE intake when consuming aspartame at the projected upper one-percentile level.

Appendix B.—Board's Decision on Potential Brain Damage From Aspartic Acid

B. Focal Brain Lesions

Since first demonstrated in 1969 by Olney and coworkers in the mouse, it has become generally recognized that the acidic, dicarboxylic amino acids glutamic acid (GLU) and aspartic acid (ASP), when present in the blood plasma in adequately high concentration, can cause death of nerve cells in the central nervous system. As far as is known at present, this neuronal necrosis is focal rather than diffuse; it is certain that it preferentially affects (1) the infundibular region of the hypothalamus, (2) the so-called circumventricular organs (the area postrema, the subfornical organ, the subcommissural organ, the vascular organ of the lamina terminalis), and (30 the retina.

The evidence that acidic amino acids are potential neurotoxins naturally has raised questions with respect to the safety of aspartame as a food additive. Roughly one half of aspartame'e molecular weight is contributed by its aspartic-acid moiety, and it is appropriate to ask whether its consumption could entail a risk of focal brain damage. Before considering the evidence it is necessary to point out that there are at least two reasons why this question concerning aspartic acid cannot be examined separately and must be considered together with a similar question concerning glutamic acid, a food additive already in wide use in the United States and elsewhere: (1) Both of these amino acids appear to be equipotential and mutually additive in their neurotoxic effects, and (2) a significant proportion of ingested aspartic acid in the course of its metabolism is transaminated to glutamic acid. For these reasons, it is the combined GLU–ASP content of blood plasma that ultimately must be considered, rather than the plasma ASP level alone. It is also for these reasons that the Board permitted a voluminous body of data concerning glutamic acid to be presented, even though aspartame itself is free from this amino acid. Throughout the following survey of data it is assumed that glutamic acid or monosodium glutamate (MSG) is exchangeable with aspartic acid or sodium aspartate in the sense that the

neurotoxic threshold levels of these substances in the blood plasma appear to be approximately the same.

Focal Brain Lesions Induced in Experimental Animals by Monosodium Glutamate

There is general agreement among investigators that high doses of MSG administered either by subcutaneous, intraperitoneal or intravenous injection, or by gavage (stomach intubation), can induce hypothalamic lesions in a variety of rodent species. Of all experimental animals used in such experiments the infant mouse, 1–10 days old, has been found most vulnerable to the neurotoxic action of MSG: a single dose of 350 mg/kg injected subcutaneously, or of 500 mg/kg administered by gavage, is enough to cause, within a few hours time, a microscopically visible lesion of the hypothalamus in about half of the infant mice so treated. Correlated with this 50%-effectiveness level of intake is a rise in plasma-GLU concentration from a baseline value of about 15 μmol/dl to 100 μmol/dl. With increasing maturity mice become more resistant to MSG: in weanling mice a 50% effect requires an MSG dose of 1200 mg/kg administered by gavage and resulting in a plasma-GLU concentration of about 380 μmol/dl. In adult mice the critical plasma-GLU concentration lies near 600 μmol/dl.

Other non-primate mammalian species seem generally less vulnerable to the neurotoxic action of MSG. Although the infant rat is nearly as sensitive to MSG as the infant mouse, the 50%-effect dose in the adult rat lies near 4000 mg/kg by gavage. The critical dose in the 2–3 day old guinea pig is about 2000 mg/kg. In dogs 3–35 days old an intake of 1100 mg/kg by gavage fails to induce hypothalamic lesions, as do doses of up to 4000 mg/kg in adult dogs.

Data for the monkey are controversial. The Board is unable to resolve the conflicts that arose over this issue at the public hearing. However, to remain on the side of safety it accepts the claims: (a) That a dose of 1000 mg/kg of MSG administered by gavage or subcutaneous injection can cause microscopically detectable hypothalamic lesions in infant monkeys ranging between prematurely born and 7 days of age, and, (b) that intravenous injection of 2000 mg/kg of MSG in the pregnant monkey can induce such lesions in her fetus. Despite existing controversies the Board also accepts the suggestion that the plasma-GLU level critical for the occurrence of hypothalamic lesions in the immature monkey lies in the vicinity of 120 μmol/dl.

MSG neurotoxicity in *pregnant or lactating animals* appears to have been studied only in a small number of species. Two separate groups of investigators have reported that in the pregnant mouse MSG must be injected in very large amounts (5000 mg/kg) to include hypothalamic lesions in her fetuses. This finding accords well with the evidence (considered in more detail below) that the placenta in the monkey maintains a highly effective barrier against both GLU and ASP only at grossly elevated maternal plasma-GLU levels (280 μmol/dl) does GLU in this mammalian species begin to enter the fetal circulation. A somewhat similar barrier appears to be maintained by the mammary gland: In the lactating human female at least, the ingestion of relatively high doses of MSG does not significantly affect the GLU content of her milk (see below).

Dietary intake of MSG by experimental animals. In all of the animal experiments mentioned in the foregoing account, MSG was either injected, or administered by stomach tube in the form of an aqueous solution. Markedly different effects upon plasma-GLU concentrations have been reported from experiments in which mice were given MSG mixed with food. Mixed with "infant formula" or with a "soup diet," and administered by stomach tube, MSG in weanling mice has been reported to induce a rise of the plasma-GLU concentration only one-fifth to one-third as large as that caused by the same amount of MSG mixed with water. Ingested by adult mice as a food additive in the enormous amount of 20,000 mg/kg, MGS has been reported to induce peak plasma-GLU concentrations no higher than 174 μmol/dl, little more than one-quarter of the plasma level (630 μmol/dl) that is correlated with hypothalamic lesions caused by subcutaneous injection of 1500 mg/kg MSG. It is relevant in this context that the archival literature includes no report of brain lesions induced in any species by dietary intake of any amount of MSG.

A postscript to these negative findings must be made. In a post-hearing communication dated April 3, 1980, to the Board and to his co-participants in the hearing, Dr. Olney reported having found clear-cut hypothalamic lesions in all of 10 weanling mice who—after having been deprived of water overnight—had drunk 0.2–0.35 ml of either a 10% aqueous GLU (presumably l-glutamic acid) solution or a solution containing 6.5% GLU, 3.5% ASP, and 1% aspartame, while concurrently consuming an unspecified amount of Purina mouse chow. The Board accepts this evidence (acknowledging that it stands at present unconfirmed) and considers that it imposes a qualification upon those statements according to which no focal brain lesions have been induced in any species by voluntary consumption of any amount of GLU or its monosodium salt. A rough calculation suggests that the weanling rats had ingested a minimum of 13 mg of GLU with the drinking water. Assuming that body weights ranged between 10 and 15 g, this intake corresponds to a loading dose of 900 mg/kg to 1300 mg/kg body weight.

Focal Brain Lesions Induced in Experimental Animals by Aspartame

In the infant mouse, 2000 mg/kg aspartame administered by gavage in the form of an aqueous slurry has been reported to cause hypothalamic lesions in 39% of subjects. No such lesions were found in any 9-day old mouse given 500 mg/kg aspartame by gavage. It seems reasonable to assume that in the infant mouse the risk of hypothalamic lesions begins to arise at a dose level of 1000 mg/kg aspartame administered by gavage. This dose approximately corresponds to 500 mg/kg aspartic acid.

Since neither the same dose nor very much higher doses of aspartame consumed by immature mice as part of the daily diet have been found to induce endocrine disorders (see below) it seems warranted to conclude that the resorption and/or metabolism of aspartic acid depends upon the route by which this amino acid is administered. Much like MSG, aspartic acid ingested as a food additive has been reported to induce elevations of the plasma-ASP level smaller than those induced by aspartic acid administered by gavage or subcutaneous injection. Further data concerning this point will be considered in a subsequent review of aspartame consumption in the human.

Neuroendocrine Disorders Induced by MSG and Aspartame in Experimental Animals

In view of the topographic characteristics of its neurotoxic effects it is not surprising that MSG administered in large amounts by subcutaneous injection has been found to induce endocrine disorders in mice, rats, and hamsters. In all of the studies from which such disorders were reported, subjects had received either a single subcutaneous injection of 3000 mg/kg MSG on the second postnatal day, or a daily injection of 2200–4000 mg/kg for 10 days starting on day 2. Prominently listed among the consequences of such treatments are: stunting of body growth, obesity, and sterility in the female.

Although apparently not explicitly demonstrated thus far, it seems reasonable to assume that in the same species subcutaneous injection of similar amounts of aspartate, or administration of aspartame by gavage in twice these amounts, would have similar endocrine consequences. It must be stressed, however, that no studies concerning the endocrine effects of subcutaneous or intragastric administration of either MSG or aspartate appear to have been done in species other than rodents. Hence, at present nothing can be said concerning the relative susceptibility of the endocrine system of various non-rodent species to parenterally administered MSG or aspartate.

Neuroendocrine Effects of Sub-neurotoxic Doses of MSG and Aspartame

One of the objecting parties has stressed the possibility that a routine intake of MSG or aspartame several times a day by children throughout their formative years could entail repetitive disturbances in several neuroendocrine axes (e.g., gonadotropins, growth hormone, and prolactin) and that such perturbations could adversely affect somatosexual development. According to this suggestion, neuroendocrine disorders induced by MSG or aspartame need not be associated with anatomically demonstrable lesions of the hypothalamus, and can be caused by an imbalance of hypothalamic function resulting from the neuroexcitatory effect of glutamate and aspartate. The notion is based upon a report by the objecting party according to which a subcutaneous injection of MSG in the presumably sub-neurotoxic amount of 1000 mg/kg in the adult rat markedly elevates plasma levels of luteinizing hormone (LH) and testosterone (TS). It was pointed out at the hearing, however, that quantitatively similar fluctuations of LH and TS levels occur normally in the course of each 24-hour period, and that the reported increases may thus have reflected no more than a normal circadian or ultradian periodicity of LH and TS release. Moreover, in two other published studies no correlation between MSG injections and fluctuations of LH and TS levels could be demonstrated.

The suggestion that a routine intake of aspartame during immature stages of development can entail an impairment of sexual function in later life would seem effectively refuted by the results of a long-term study of the effects of aspartame consumption on reproductive function in the rat. In this study, a daily dietary intake of very large amounts of aspartame ranging between 1800 and 3700 mg/kg, beginning on postnatal days 10–20 and ending on days 90–100, did not affect fertility, gestation, live birth, litter size, or nursing in either the experimental subjects or their offspring. The results of several further studies presented at the hearing likewise indicate that endocrine disorders are induced by MSG only when this substance is administered in amounts large enough to cause identifiable hypothalamic lesions. The experimental evidence thus appears to argue against the notion of sub-neurotoxic effects upon the neuroendocrine axis.

Glutamate and Aspartame Consumption in the Human

Among the data presented on this subject, the Board considers the following pragmatic evidence of particular relevance.

1. *In the adult,* a loading dose of 34 mg/kg aspartame (the 99th percentile of a projected mean daily consumption of 7–9 mg/kg, and roughly equivalent to 100 tablets of 20 mg aspartame) dissolved in orange juice induces no significant elevation of either plasma GLU or plasma ASP concentration. Neither does a loading dose of 50 mg/kg aspartame induce any significant rise of GLU or ASP concentration in either blood plasma or erythrocytes.

2. A similarly administered aspartame loading dose of 200 mg/kg in the adult (equivalent to 600–800 aspartame tablets) causes the plasma ASP level to rise from a baseline of 0.2 μmol/dl to 1 μmol/dl, receding to baseline in 3 hours. Following such a dose, the plasma GLU level rises from 2.5 μmol/dl to 6 μmol/dl for a combined plasma GLU+ASP rise to 7 μmol/dl.

3. A hamburger-milkshake meal providing 1 g of protein per kg body weight, and containing free plus protein-bound GLU in the amount of 171–198 mg/kg body weight and free plus protein-bound ASP in the amount of 90–103 mg/kg body weight, causes an elevation of the plasma GLU level from a baseline of 4 μmol/dl to 9 μmol/dl, and raises the plasma ASP level from a baseline of 0.3 μmol/dl to 0.8 μmol/dl. The addition of 34 mg/kg MSG (the 90th percentile of projected MSG consumption) to this meal has no effect upon these post-prandial elevations, and neither does the addition to the meal of 34 mg/kg MSG plus 34 mg/kg aspartame. If the MSG addition to the meal is increased to 150 mg/kg the plasma GLU+ASP level rises from a baseline of 5 μmol/dl to 25 μmol/dl; the addition of 34 mg/kg aspartame in this case causes no further increase in the plasma GLU+ASP level.

4. *In one-year old infants,* a loading dose of 100 mg/kg aspartame induces a rise of the plasma ASP level from a baseline of 1.5 μmol/dl to 2.6 μmol/dl, receding to baseline in 1–2 hours. This finding appears to refute any suggestion that aspartic acid might be metabolized less efficiently in infants than in adults.

5. *In PKU heterozygote* adults aspartame loading doses of 34 mg/kg and 100 mg/kg are metabolized much as they are in normal individuals. The resulting rise in plasma GLU level is virtually the same in both categories of subjects, while the rise in plasma ASP level is slightly, but not significantly, higher: Plasma GLU+ASP level reaches a mean of 4.5 μmol/dl in normal adults, a mean of 4.8 μmol/dl in PKU heterozygote adults.

6. *In the lactating woman,* a loading dose of 50 mg/kg aspartame (about 150 aspartame tablets) induces no significant elevation of plasma ASP or GLU levels. This dosage raises the ASP concentration in her milk from 2.3 to 4.8 μmol/dl, the GLU concentration from 109 to 120 μmol/dl. At this high level of maternal aspartame intake, the breast-fed infant's normal daily intake of 366 mg/kg GLU+ASP is increased by no more than 0.77 mg/kg.

7. *Placental transfer of ASP to the fetus.* For obvious reasons, this problem cannot be directly approached experimentally in the human. The following conclusions are based upon experiments in pregnant monkeys.

The primate placenta maintains a 1:2 plasma-concentration gradient toward the fetal circulation for most amino acids. However, both GLU and ASP are exceptions to this rule. GLU is not transferred at all from the maternal to the fetal circulation even when the maternal plasma level is increased from a baseline of 5 μmol/dl to 55 μmol/dl; only at the enormously elevated maternal plasma GLU level of 280 μmol/dl—induced by direct intravenous infusion of GLU—does some transfer to the fetus take place. The placenta maintains an equally effective barrier against ASP: intravenous infusion of 100 mg/kg ASP (in one hour) elevates the maternal ASP level from a baseline of 0.4 μmol/dl to 80 μmol/dl; the fetal plasma ASP level under these conditions does not exceed 0.42 μmol/dl. Maternal ASP infusion of 200 mg/kg/hr induces a maternal plasma ASP rise to 237 μmol/dl, while the fetal plasma ASP level rises from a baseline of 0.6 μmol/dl no further than 4.5 μmol/dl.

Taken together with items 1 and 2 above, these findings indicate that both mother and fetus are thoroughly protected against hazardous plasma

ASP levels: The mother by a highly effective barrier of ASP resorption and/or metabolism, the fetus in addition by an equally effective placental barrier. The mother herself has no comparably effective defense against GLU, but plasma GLU levels high enough to place her at risk are not reflected in the fetal blood plasma.

Risk Evaluation

In attempting to assess the risk of focal (in particular, hypothalamic) brain damage connected with human aspartame consumption, the Board decided to adopt a 100 μmol/dl concentration of GLU+ASP in the blood plasma as the critical level. This conservative assumption was made for reasons of caution: 100 μmol/dl is the concentration at which a 50% occurrence of focal brain lesions has been reported for the infant mouse, the animal form generally thought to be most sensitive to the neurotoxic effects of glutamic and aspartic acid. the problem thus became reduced to the question whether, and at what level of consumption by the human aspartame could induce plasma GLU+ASP elevations approaching the 100 μmol/dl level when taken alone, or alternatively, whether it could significantly contribute to such elevations induced by MSG consumption. It should be recalled in this connection that—unlike the brain damage associated with phenylkalanine—the focal brain lesions associated with GLU and ASP neurotoxicity are not contingent upon a long-maintained high plasma concentration of the causative agent: It is evident from animal experiments that focal hypothalamic lesions can be induced by a single elevation of the plasma GLU and/or ASP concentration to the level of 100 μmol/dl.

It is of some historic interest that much of the evidence reported to the Board concerning the aforementioned question dates from recent years (1976–1979), and consequently was not available at the time the objections to the approval of aspartame as a food additive were originally filed. With a single exception, the following statements can at present be considered justified by the results of experiments done directly in the human rather than in one or more animal species:

1. The human organism, infant as well as adult, is protected against high surges of ASP concentration in either blood, plasma or erythrocytes by a biological barrier mechanism presumably located in the gastrointestinal mucosa and/or liver. The effectiveness of this protective mechanism is illustrated by the observation that loading doses of aspartame as high as 200 mg/kg body weight (in a 60 kg individual equivalent to 600 aspartame tablets or 20 liters of aspartame-sweetened beverage consumed in a single sitting) induce an elevation of plasma and erythrocyte GLU+ASP concentration of no more than 5 μmol/dl above a baseline level of 2.5–3 μmol/dl. It is of added significance that these elevations are short-lived, receding to baseline in about 3 hours time. It follows that repeat-doses of the same enormous magnitude, when spaced 3 hours apart, are unlikely to escalate the GLU+ASP concentration much beyond the level induced by the first dose.

2. The ASP plasma-entry barrier is unaffected by simultaneously ingested MSG: the 25 μmol/dl plasma GLU+ASP concentration achieved by adding to a protein-rich meal a very large dose of MSG (150 mg/kg, or 9000 mg in the case of a 60 kg person) is not augmented by the further addition of 34 mg/kg aspartame (100 aspartame tablets) to the meal.

3. The PKU heterozygote adult is no less effectively protected against aspartame-induced surges of plasma GLU+ASP concentration than the normal human.

4. In the breast-fed infant, a consumption of 50 mg/kg aspartame by the lactating mother results in an increase of no more than 0.77 mg/kg GLU+ASP over the normal daily intake of 366 mg/kg GLU+ASP

5. The speculation that aspartame consumption by the pregnant women could expose her fetus to a high risk of focal brain damage cannot be investigated directly in the human. However, experimental findings in the monkey indicate that the primate placenta maintains a nearly insurmountable barrier against any transfer of GLU and ASP from the maternal to the fetal circulation.

Conclusion Regarding Aspartame-Induced Focal Brain Lesions

In the Board's opinion, the most pertinent evidence presented at the public hearing convincingly demonstrates that *the risk of focal brain damage associated with aspartame consumption in the human is negligible.* Elevation of plasma GLU+ASP concentration even to the lowest level that could be suspected of being neurotoxic (100 μmol/dl) would require an inconceivably high oral aspartame intake. Such levels might in fact prove attainable only by parenteral ASP administration designed to bypass the highly effective intestinal and/or hepatic barrier mechanism guarding against surges of plasma ASP concentration.

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