CENTER FOR
Science IN THE
Public Interest
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TO: Division of Dockets Management (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Room 1061
Rockville, MD 20852

Re: Docket No. FDA-2009-F-0303

The Center for Science in the Public Interest (CSPI) submits these comments on the final rule issued May 21, 2014 approving the use of advantame in foods.[1]

We believe that two key studies relied upon for the safety evaluation of advantame were significantly flawed and provide an inadequate basis for ensuring safe use of the ingredient. We are concerned by the agency's failure to abide by its own published standards for the safety assessment of food ingredients, as well as by its dismissal of concerns raised by certain agency scientists regarding the adequacy of studies for an additive that will likely be consumed by millions of people. While we are not filing a formal objection, we urge the FDA, when it evaluates future additives, to require that safety studies meet the standards and principles the Agency has set forth for this purpose; make judgments that reflect public health concerns; request additional data or other information from petitioners when there are information gaps or uncertainties; and, in short, set a higher safety bar to ensure that there is convincing evidence that establishes with reasonable certainty that no harm will result from the use of additives in order to ensure public health protection and public confidence in agency actions.

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[1] "Food Additives Permitted for Direct Addition to Food for Human Consumption: Advantame; Final Rule," 79 Federal Register 98 (May 21, 2014), pp. 29078-29085 (hereinafter refered to as the Federal Register notice). Section 172.803(c) states:

> The food additive advantame may be safely used as a sweetening agent and flavor enhancer in foods generally, except in meat and poultry, in accordance with current good manufacturing practice, in an amount not to exceed that reasonably required to achieve the intended technical effect, in foods for which standards of identity established under section 401 of the Federal Food, Drug, and Cosmetic Act do not preclude such use.

A careful scientific review is required by both the safety standard for food additives specifying that a "proposed use of the food additive, under the conditions of use to be specified in the regulation, will be safe." *See* 21 U.S.C. § 358 (c) (3)(A). In addition, the so-called "Delaney Clause" requires that "no additive shall be deemed to be safe if it is found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal." *See id.*

Specifically, our review revealed the following flaws and inadequacies:

1) **The carcinogenicity study in mice failed to meet FDA's own recommended standards for survival of animals.** FDA's "Toxicological Principles for the Safety Assessment of Food Ingredients – Guidance for Industry and Other Stakeholders," known as the "Redbook," states in Chapter IV.C.6, on "Carcinogenicity Studies with Rodents," (January 2006) section II.D "Number and Sex," that "Experimental and control groups should have a sufficient number of animals at the beginning of the study to ensure that at least 25 **r**odents per sex per group survive to the end of the study." However, only one of eight groups met that standard: the female controls. None of the male groups met that standard. So many mice died in the advantame study that in several groups, fewer than 20 animals remained alive at 104 weeks. Only 17 animals of the original 64 (27%) were still alive at week 104 among females given the highest dose of advantame and among males given the lowest dose of advantame; and only 15 of 64 females (23%) given the lowest dose of advantame survived to the end of the study (104 weeks), as noted in Reference 13, Memorandum from I. Chen, May 24, 2010. As indicated in a memo by a scientist on the Toxicology Review Team (reference 15 of the docket), the survival range is "inconsistent with CFSAN Redbook guidance (revised 2006) which *requires* [emphasis added] 25 animals alive in each dose group at [the] end of the treatment period in a carcinogenicity study." The memo states "Because of the large numbers of deaths per group in this study, DPR Toxicology [FDA Division of Petition Review, Toxicology Review Group] was uncertain whether data from the 104-week mouse study could be utilized to assess the carcinogenic potential of advantame in mice."

    a. **FDA's own biostatistician expressed concerned that the reduced survival in mice in the chronic study compromised the ability of the study to detect late-developing tumors.** The memo from the CFSAN Cancer Assessment Committee (CAC) Full Committee Review (April 27, 2012, listed as reference 16) states "the CFSAN Biostatistician concluded that the reduced survival and increased number of deaths occurring between weeks 80 and 104, especially in the low and high dose female mice groups compared to control group, *probably masked the occurrence of late developing tumors* (CFSAN Biostatistics Branch, Dr. D. Ruggles, memo dated 03/02/2012) [emphasis added]." In particular, we note the dose-response increase in bronchio-alveolar adenocarcinomas that showed a

significant increase at the low dose and borderline significant increase at the mid- and high-dose levels. The FDA biostatistician noted, "if 25 or more animals survived to sacrifice there would have been a great likelihood that more of these tumors would have occurred in the treated groups compared to the control resulting in significant finding instead of borderline significant finding."

b. **The Cancer Assessment Committee (CAC) did not provide any substantive basis or scientific rationale for disregarding the Redbook standards or the advice of the Biostatistician, both of which are well grounded.** The CAC stated, "While the CAC acknowledged these findings, the CAC still believed that the data in this mouse study were adequate to evaluate the carcinogenic potential of Advantame."

c. **The basis provided in the Federal Register notice for determining that the mouse study is acceptable for evaluating the carcinogenicity of aspartame despite the low survival rate is not compelling and includes statements that contradict statements by agency scientists.** FDA states in the Federal Register notice:

> We noted a low survival rate of the test animals, a common finding in 2-year bioassays using the CD-1 mouse, and a number of various clinical signs in both the control and treated mice (Ref. 13). Our evaluation of the mouse survival data revealed no evidence of premature deaths that were due to treatment and none of the findings indicated a proliferative response as the cause of early death in these mice. We considered the data available up to the 92-week observation period and determined that 25 or more surviving animals per group was adequate to evaluate the carcinogenic potential for advantame. We concluded that none of the clinical signs observed correlated consistently with a histomorphological diagnosis or were an indication of treatment-related toxicity (Ref. 14).

However:

(1) Survival of the test animals was lower in this study compared to average survivorship rates for CD-1 mice. Survival data were recently compiled[2] for CD-1 mice in nine carcinogenicity studies of two years duration; the terminal mean survival rates was 45% in males and 43% in females at terminal sacrifice. Similarly, the mean survival rate was 41% for males and 37% for females in a compilation of 14 104-week studies using CD-1 mice.[3]

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[2] Le Bigot, JF, Thirion-Delalande, C, Palate, B, Forster, R. Lifetime carcinogenicity studies in the CD-1 mouse: historical data for survival and neoplasms. CiTox LAB, France. Poster SOT 2014. http://www.citoxlab.com/wp-content/uploads/2014/04/Lifetime-carcinogenicity-studies-in-the-CD1-mouse-neoplasms.pdf

[3] Giknis, MLA, Clifford, CB. Spontaneous Neoplastic Lesions in the Crl:CD-1 (ICRR) Mouse in Control Groups from 18 Month to 2 year Studies. Charles River Laboratories. March 2005

The survival rate in this study was 31% in control males at week 104 and 41 percent in control females, with only 23-38% of treated animals surviving to week 104. The reason for the low survival in this study was not made clear.

(2) The statement "… no evidence of premature deaths that were due to treatment" contradicts the conclusions of Agency scientists that found significant differences between treated and untreated females. As stated in reference 15, Attachment 4, survival for the female controls was significantly greater (p=0.029) vs. the low dose and borderline significantly greater (p=0.067) vs. the high dose. The FDA biostatistician stated, "Between weeks 80-90, 5 of the 40 (12.5%) surviving control mice died compared to 12/39 (30.8%) **p = 0.044 for the low dose,** 7/36 (19.4%) p>0.1 for the mid dose, and 13/41 (31.7%) **p = 0.034 for the high dose** [emphasis added]." Similar results are provided between weeks 80-96. FDA's Dr. Chen explained, "after week 80 significant numbers of the treated female mouse were dead as compared to the control group."

(3) The statement "none of the early deaths were caused by a proliferative response" is not very relevant or compelling. Proliferative responses do not need to be the cause of premature death to be relevant. Non-fatal proliferative responses might have progressed, or developed, had the animals lived longer. In fact, that point is made in the memo by the FDA biostatistician cited above. There were no interim sacrifices conducted, which might have shed additional light on early development of proliferative responses.

(4) A 92-week observation period is less sensitive at detecting late-developing tumors than 104 weeks. CSPI and others have expressed concern that even 104 week studies may be too short to detect cancers that arise in old age. A further shortening of the observation period further decreases the sensitivity of the study.

2) **The carcinogenicity study in mice may have been compromised, as evidenced by the high incidence of unusual, extreme, and unexpected clinical signs.** The memo from the CFSAN Cancer Assessment Committee (CAC) Full Committee Review (April 27, 2012, listed as reference 16) notes the "high incidences of a number of abnormal clinical signs." Those included "abnormal behavior (e.g., aggression, irritability, overactivity, underactivity, vocalization, circling, salivation), incidences of abnormal muscle reactions (e.g., convulsion, tremor, fasciculation, reduced body tone, abnormal gait, limited use of limbs; incidences of abnormal posture (e.g., hunching, tilted righting

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(provided by Eyassu Abegaz, Scientific and Regulatory Affairs, Ajinomoto North America, June 19, 2014).

reflex); incidences of abnormal eye movement, partially closed eye lids, pupil dilation and incidences of abnormal respiration (e.g., deep breathing, shallow breathing, irregular breathing, slow breathing and gasping)." Although there was not a difference in these abnormal signs between controls and treated animals, clearly those are not symptoms that should be occurring in healthy animals. The symptoms apparently occurred sporadically, and there is no mention of them being confined only to very old animals. Were there lapses in animal husbandry that corresponded to the timing of such symptoms? Did the laboratory not maintain proper temperatures for the animals? Were the animals exposed to another chemical that might have caused these neurological and other symptoms? The presence of that variety of unexpected symptoms would seem to call into question the reliability of the entire study and the adequacy of the practices at the facility where the study was conducted; that they affected both treated and untreated animals does not alter that concern.

3) **As noted by Agency scientists, the reliability of the carcinogenicity study in rats appears to have been compromised because weaker and abnormal rats from the *in utero* phase were excluded from the study, leading to biased outcomes (toward the null hypothesis or away from finding adverse effects).** The memo from the CFSAN Cancer Assessment Committee (CAC) Full Committee Review (April 27, 2012, listed as reference 16) states,

> The experimental design of the Advantame rat carcinogenicity study was determined to be adequate and acceptable by the CFSAN CAC *except for the practice of culling* to achieve litter sizes of 8 based on removing the weaker and abnormal rats from the *in utero* phase of the study. *The CAC strongly objected to this practice*. They discussed that the practice of removing the weaker/abnormal animals to achieve litter sizes of 8 *may have compromised and confounded the outcome of the results* of the Advantame treatment in the in utero *testing phase and biased the animal selections for the main carcinogenicity study*. In place of this practice, the Committee recommended that the culling procedure should be based on a randomization selection procedure across groups. [emphasis added]

Weaker and abnormal animals might not be as strong in defending against carcinogenic or other toxic effects. A properly conducted study might have provided stronger evidence of carcinogenicity or toxicity, or lack thereof. Culling to remove weaker animals is analogous to the "healthy worker effect" seen in populations of healthy workers, compared to the general population. It is disappointing that the FDA accepted this study despite the strong objections of some of its own scientists.

4) **Despite the bias of the carcinogenicity study in rats away from finding adverse effects, the study found evidence of a carcinogenic response; FDA's arguments for**

dismissing tumors in the test groups and concluding that the study demonstrated a lack of carcinogenicity are not compelling.

a. **Mammary adenomas in female rats in the high-dose group were significantly higher than that of concurrent controls and outside the historical control range.** The memo from the CFSAN Cancer Assessment Committee (CAC) Full Committee Review (April 27, 2012, listed as reference 16) includes Attachment 1, a memorandum from Sabine Francke-Carroll, DVM, PhD, FIATP and Steven Mog, DVM, DACVP, Senior Science and Policy Staff, Office of Food Additive Safety dated May 16, 2012, states,

> We agree with the study authors based on the data provided in their narrative the incidence distribution of mammary adenomas (0, 0, 0, 4) *appears elevated (9.8%) over the concurrent control group*. *In addition*, when considered alone, mammary gland adenomas of the high dose (50000 ppm) *fell clearly outside the cited* (Text Table 12) *historical control data range (0-2%)* of the Huntingdon Life Sciences study laboratory (Volume 1, pg 12). *Furthermore* mammary gland adenomas are *generally considered 'uncommon*' compared to other mammary gland tumors (fibroadenomas and adenocarcinomas) as reported in the published literature (Poteracki *et al*., 1998; Walsh *et al*., 1994 and Bomhard *et al*., 1986). These authors reported mammary adenomas within the following incidence ranges 2-6.7%, 0-8%, 0-2.2% respectively. [emphasis added]

b. **The total epithelial proliferative changes in the female mammary gland in the high-dose group is increased compared to controls, and there is a dose-responsive upward trend.** The same May 16, 2012, memo cited above notes,

> Adenomas, by convention, represent a continuum of proliferative changes ranging from acinar hyperplasia to adenoma to adenocarcinoma. Therefore, the initial analysis should first establish if there is presence or absence of a treatment related proliferative continuum toward malignancy based on the evaluation of individual female rats with mammary epithelial cell proliferative data (acinar hyperplasia, adenoma, adenocarcinoma)….

The memo then includes Table 2 which shows that the combined proliferative response shows a dose-response trend: 11.6%, 18.2%, 19.0%, 31.7%. Later it states, after adjusting the reported results, "when the total epithelial proliferative response (hyperplasia, adenoma, adenocarcinoma) is

evaluated**,** *a dose responsive upward trend is apparent, especially in the high dose (23.6%)* [compared to 9.1% in controls; emphasis added]."

c. **The evaluation and evidence provided is not sufficient to conclude that this is a negative study for carcinogenicity.** There were no interim sacrifices to judge whether proliferative changes might have occurred earlier in treated animals compared to controls. While it is true that there is no dose-response trend in the severity of hyperplasia, that is not sufficient reason to dismiss the uncommonly high rate of adenomas in the high-dose females, the non-significant increase in adenocarcinoma in the high-dose females, and the dose-responsive upward trend of the combined proliferative response. Furthermore, given the low historical control rate of mammary gland adenomas in the Hans Wistar rat, this is likely not a sensitive model for the evaluation of mammary neoplasms. At the very least, to ensure safety, advantame should be re-tested in a more appropriate strain of rat.

d. **The culling of weaker animals might have affected rates of non-mammary tumors.** Had the rat study included more of the weaker animals culled from the study, the pancreatic islet-cell carcinomas that showed a dose-responsive trend that was just shy of significance (p=0.06) might have achieved statistical significance.

Sincerely yours,

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