Consideration of the Prioritization of Aspartame

Comments of the Calorie Control Council, American Beverage Association and National Confectioners Association to the Proposition 65 Carcinogen Identification Committee

October 24, 2016

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I. Summary

The Calorie Control Council, American Beverage Association and National Confectioners Association respectfully recommend that the Carcinogen Identification Committee (CIC) maintain aspartame's current priority level, which is "at the bottom of the medium category." Aspartame's priority level should not be elevated for the following reasons.

The FDA (2014), European Food Safety Authority (EFSA, 2013), and other health authorities have repeatedly and recently reviewed all of the carcinogenicity data concerning aspartame and consistently found no cause for concern. In fact, no regulatory agency in the world considers aspartame to be a carcinogen.

There is only one animal carcinogenicity study (Soffritti et al., 2010) that has become available since the CIC's "bottom of the medium category" prioritization of aspartame. According to the European Food Safety Authority (EFSA, 2013), "the results of the studies performed by Soffritti et al. (2010) do not provide evidence for a carcinogenic effect of aspartame in mice." There are three epidemiologic studies that were published after 2009: (1) a "weak" positive prospective cohort study (Schernhammer et al. 2012) where even the authors cannot rule out "chance" as an explanation for some effects, (2) a negative prospective cohort study (McCullough et al., 2014), and (3) a negative case control study (Cabaniols et al., 2011). None of these four new studies warrant elevating aspartame's priority level.

In total (since 1973), ten carcinogenicity studies of aspartame have been conducted in rats or mice (see Table 1). Aspartame was not carcinogenic in seven of these studies, including three studies by the National Toxicology Program (NTP, 2005). The only evidence of carcinogenicity in animals comes from three unconventional and highly controversial studies conducted by Soffritti et al. (2006, 2007, 2010) at one laboratory (Ramazzini Institute). These studies reported an increased incidence of combined leukemia/lymphoma in rats and of liver and lung tumors in male mice. However, the Ramazzini studies have multiple, serious deficiencies in experimental design, interpretation of results and data reporting (Magnuson et al., 2007; Schoeb et al., 2009; NTP, 2011; EFSA, 2011, 2013; EPA, 2012; FDA, 2014). EPA recently concluded that many of the malignant neoplasms in rat carcinogenicity studies by Ramazzini have been misdiagnosed and are actually hyperplasias related to unknown chronic infection in the animals. No regulatory agency has considered the Ramazzini aspartame studies to be credible evidence of carcinogenicity, and no regulatory agency has ever relied upon the Ramazzini studies of aspartame for regulatory purposes. In addition, the genotoxicity studies provide no basis to elevate the priority level for aspartame.

In humans, the potential carcinogenicity of aspartame has been evaluated in three prospective cohort studies and three case-control studies (see Table 2). The only evidence of carcinogenicity in humans is a "weak" positive in one of the prospective cohort studies where even the authors say the results "necessarily require confirmation" ("chance" cannot be ruled out) in other prospective cohort studies (Schernhammer et al., 2012). But, no increased risk of cancer attributable to aspartame was identified in the other two prospective cohort studies (Lim et al., 2006; McCullough et al., 2014). The three case-control studies provide additional support for the lack of a positive association between aspartame consumption and cancer risk.

II. Introduction and Background

Aspartame, a synthetic non-nutritive sweetener, has been approved for use in food in more than 100 countries, including the U.S., Canada, the European Union (EU) and its member states, Japan, and Australia. No country has declined to approve the use of aspartame in food. And, no regulatory agency considers aspartame to be a carcinogen. In 2014, FDA "concluded that consumption of aspartame is well below the acceptable daily intake [FDA ADI: 50 mg/kg bw/day], that it is safe for its intended use, and that high levels of human aspartame intake are unlikely to exceed the ADI when it is used in food under current good manufacturing practice."[1]

Aspartame was first prioritized by the CIC at its May 29, 2009 meeting. The CIC recommended that aspartame be placed "at the bottom of the medium category."[2] Based on its recent notice, OEHHA is now asking the CIC to prioritize five substances, including aspartame, at its November 15, 2016 meeting. Aspartame is being brought back to the CIC for consultation because, according to the OEHHA data summary: "Since 2009, additional epidemiology data, animal cancer bioassays, and genotoxicity data have become available."[3]

The purpose of this document is to describe briefly the limited number of additional studies of aspartame that were not available to the CIC in 2009, as well as the recent reviews of aspartame by regulatory agencies. Neither the additional studies nor the agency reviews of aspartame indicate that the CIC should raise aspartame's priority level.

III. Only one additional animal carcinogenicity study has become available since 2009, and it does not provide a basis to elevate the priority level for aspartame

Table 1 contains a summary of all of the available animal carcinogenicity studies of aspartame. OEHHA's data summary of aspartame indicates that four additional animal carcinogenicity studies have been identified as "additional references identified since prioritization and consultation in 2009." This is incorrect. There is only one additional animal carcinogenicity study that was not considered by the CIC in 2009: the Soffritti et al. (2010) carcinogenicity study in mice conducted at the Ramazzini Institute. This study is described briefly below.

The other three studies described in OEHHA's aspartame data summary as "additional references identified since prioritization and consultation in 2009" were not included in the 2009 OEHHA data summary of aspartame.[4] However, all three of these studies were, in fact, considered by the CIC in 2009; they were described in detail in the written comments to the CIC

[1] FDA (2014) Letter from Steven Musser, Ph.D., Deputy Director for Scientific Operations, Center for Food Safety and Applied Nutrition (CFSAN), FDA to Mrs. Betty L. Martini. Citizen Petition Denial. Docket No. FDA-2002-P-0247. Oct. 1, 2014, p. 4.
[2] Transcript of May 29, 2009 meeting of the CIC, p. 89.
[3] OEHHA (2016) Chemical for CIC Consultation: Aspartame. August, 2016.
[4] The other three studies described "as additional references identified since prioritization and consultation in 2009" are Searle, E70 (1974), Searle, E-75 (1974), and Ishii et al. (1981).

by the Calorie Control Council several weeks prior to the 2009 CIC meeting.[5] All three of these animal carcinogenicity studies are described in the current OEHHA data summary as negative studies, and as such they do not provide a basis for elevating aspartame's priority level.

Soffritti et al. (2010)

This study adds little to the weight of the evidence of carcinogenicity because of serious concerns with the experimental design, lack of dose-response, statistical analyses and interpretation of results. Regulatory agencies have been highly critical of this study, and to the best of our knowledge, the conclusions of this study have never been accepted by any regulatory agency in the world. Soffritti et al. (2010) conducted a long-term feeding study of aspartame in Swiss mice. Exposure started prenatally on gestation day 12 and continued through lifetime until death; there was no scheduled sacrifice at 2 years or any other time. According to Soffritti et al. (2010), significant increases in liver tumors (pair-wise comparison) and lung tumors (trend test only) were observed in males, but not females.

The carcinogenicity studies conducted at the Ramazzini Institute have been the subject of considerable controversy and criticism, and the Soffritti et al. (2010) study is no exception. The European Food Safety Authority (EFSA) evaluated this study in 2011 and offered the following opinions:

> "EFSA has evaluated the carcinogenicity study with transplacental exposure to aspartame as reported by Soffritti et al. (2010). EFSA concluded that, on the basis of the information available in the publication, the validity of the study and its statistical approach cannot be assessed and its results cannot be interpreted. Furthermore, in view of the generally recognised lack of relevance for human risk assessment of the tumours observed in Swiss mice when they are induced by non-genotoxic compounds, EFSA concluded that the results presented in the publication by Soffritti et al. (2010) do not provide sufficient scientific evidence to reconsider the previous evaluations by EFSA on aspartame that concluded on the lack of genotoxicity and carcinogenicity of the sweetener."[6]

EFSA (2011) also noted that "it is generally accepted that life time studies until or close to natural death can lead to erroneous conclusions because of the following limitations. Older animals are more susceptible to illness and have increased background pathology, which includes spontaneous tumours and have a higher probability of autolysis than younger animals."[7]

In addition, the French Agency for Food, Environmental and Occupational Health and Safety (ANSES, 2011) made the following assessment regarding Soffritti et al. (2010):

> "On the study by Soffritti et al.: the study was carried out using Swiss mice, according to an unusual protocol that does not comply with the international guidelines laid down for

[5] F. Jay Murray (2009) Comments of the Calorie Control Council to the California Carcinogen Identification Committee. May 5, 2009. pp. 8, 16.
[6] EFSA (2011) Statement of EFSA on the scientific evaluation of two studies related to the safety of artificial sweeteners. p. 13.
[7] Id., p. 2.

this type of study. It is generally accepted that in this type of experimental protocol the physiological condition of the animals declines substantially with age, which can distort the results and cast doubt on the conclusions of the study. Furthermore, the incidences of

Table 1. Summary of Animal Carcinogenicity Studies of Aspartame

Study	Species	Dose, g/kg bw/day	Duration	Results	Comments	Considered by CIC in 2009?
Searle, E33/34 (1973)	Sprague-Dawley CD Rat	0, 1, 2, 4, 6-8	2 yr	Not carcinogenic	Regulatory submission to FDA	Yes
Searle, E70 (1973)	Sprague-Dawley CD Rat	0, 2, 4	In utero thru 2 yr	Not carcinogenic	Regulatory submission to FDA	Yes
Searle, E75 (1973)	ICR Swiss Mouse	0, 1, 2, 4	2 yr	Not carcinogenic	Regulatory submission to FDA	Yes
Ishii et al. (1981)	SLC Wistar Rat	0, 1, 2, 4	2 yr	Not carcinogenic	Regulatory submission to FDA	Yes
NTP (2005)	Heterozygous p53-deficient Mouse	0, 0.5, 1, 2, 4, 8	9 mo	Not carcinogenic	Sensitive for lymphomas and sarcomas	Yes
NTP (2005)	Cdkn2a-deficient Mouse	0, 0.5, 1, 2, 4, 8	9 mo	Not carcinogenic	Sensitive for brain tumors	Yes
NTP (2005)	Tg.AC Mouse	0, 0.5, 1, 2, 4, 8	9 mo	Not carcinogenic	Sensitive for genotoxic/ nongenotoxic carcinogens	Yes
Soffritti et al. (2005, 2006)	SD Rat (institutional colony)	0, 0.004, 0.02, 0.1, 0.5, 2.5, 5	Thru spontaneous death	Positive[a]	Seriously flawed. FDA, EFSA and others disagreed with conclusions.	Yes
Soffritti et al. (2007)	SD Rat (institutional colony)	0, 0.06, 0.3	In utero thru spontaneous death	Positive[b]	Seriously flawed. FDA, EFSA and others disagreed with conclusions.	Yes
Soffritti et al. (2010)	Swiss mouse (institutional colony)	0, 0.25, 1, 2, 4	In utero thru spontaneous death	Positive[c]	Seriously flawed. FDA, EFSA and others have not accepted the conclusions	No

[a] Increase in combined lymphoma/leukemia in females, renal carcinomas, malignant schwannomas of peripheral nerves
[b] Increase in (a) malignant tumors in males, (b) lymphoma/leukemia in males & females, (c) mammary cancer in females
[c] Increase in hepatocellular and alveolar-bronchiolar carcinoma in males, but not females.

> liver and lung tumours reported in this study are characteristic of, and frequently observed to occur spontaneously in, the strain of mice studied. The statistical analyses carried out do not show a dose-effect relationship for aspartame. In addition, because of the uncertainties and methodological deficiencies, it is impossible to characterize the effects reported in this study so as to extrapolate them to the situation in humans."[8]

Finally, FDA has not accepted the results of this study. FDA requested data from this study from the authors, but the authors have not complied with this request. To date, it is our belief that no regulatory agency in the world has relied upon the results of this study.

IV. The three new epidemiologic studies since 2009 provide no reason to elevate the priority level for aspartame.

When aspartame was prioritized in 2009, five epidemiological studies of aspartame and/or diet soda consumption were identified and considered by the CIC: two ecological studies, two case-control studies, and one prospective cohort study. Since 2009, two additional prospective cohort studies and one additional case-control study have been published, as summarized in Table 2. Overall, these three studies, described briefly below, provide no reason to elevate aspartame's priority level.

Schernhammer et al. (2012)

Schernhammer et al. (2012) at Brigham and Women's Hospital published a well-conducted prospective cohort study of aspartame consumption in soda and hematopoetic cancers in 77,218 women from the Nurses' Health Study and 47,810 men from the Health Professionals Follow-Up Study in the U.S. The authors concluded: "Although our findings preserve the possibility of a detrimental effect of a constituent of diet soda, such as aspartame, on select cancers, the inconsistent sex effects and occurrence of an apparent cancer risk in individuals who consume regular soda ***do not permit the ruling out of chance as an explanation***." [emphasis added] The OEHHA data summary notes that there were several statistically significant associations between non-Hodgkins lymphoma (NHL) and multiple myeloma among men, but not among women. A similar association with NHL was also observed with ***sugar-sweetened*** soda among men, but not women. An association between leukemia and diet soda consumption was reported among men and women combined, but not among men or women considered separately.

The authors note that their study is "the first large-scale observational human study to report associations between diet soda and aspartame intake and these cancer types." Schernhammer et al. (2012) acknowledged: "our results necessarily require confirmation in other large cohorts."[9] So far, these findings have not been confirmed by either of the other two large-scale prospective cohort studies of aspartame, including the more recent study by McCullough et al. (2014) described below.

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[8] ANSES (2011) Opinion of the French Agency for Food, Environmental and Occupational Health & Safety on a publication reporting the incidence of cancer in male mice after administration of aspartame in their feed. ANSES – Request No. 2011-SA-0015. March 14, 2011.

[9] Schernhammer et al. (2012) p. 1427.

Table 2. Summary of the epidemiological cancer studies of aspartame

Epidemiologic Studies	Type of Study	Result	Considered by CIC in 2009?
Roberts (1991)	Ecological	Temporal association (brain tumors increased after aspartame introduced)	Yes
Olney (1996)	Ecological	Temporal association (brain tumors increased after aspartame introduced)	Yes
Gurney et al. (1997)	Case-control	No association (brain cancer)	Yes
Gallus et al. (2007	Case-control	No association (10 cancer types)	Yes
Cabaniols et al. (2011)	Case-control	No association (brain tumors)	No
Lim et al. (2006)	Cohort, prospective	No association (brain tumors, leukemia, lymphoma)	Yes
Schernhammer et al. (2012)	Cohort, prospective	Weak & inconsistent associations (NHL, multiple myeloma)[a]	No
McCullough et al. (2014)	Cohort, prospective	No association (NHL)[b]	No

[a] "Conclusion: Although our findings preserve the possibility of a detrimental effect of a constituent of diet soda, such as aspartame, on select cancers, the inconsistent sex effects and occurrence of an apparent cancer risk in individuals who consume regular soda do not permit the ruling out of chance as an explanation."
[b] Conclusion: "These findings do not support associations of daily consumption of artificially or sugar-sweetened carbonated beverages, or aspartame, with NHL risk." Some associations with NHL were observed in Quintiles 2 and 3, but not in Quintiles 4 and 5.

Notably, when the results of this study were first released, the Brigham and Women's Hospital's Media Relations issued a press release that sensationalized the study findings. Subsequently, Brigham and Women's Hospital released a statement to provide perspective: "Upon review of the findings, the consensus of our scientific leaders is that the data is weak, and that BWH Media Relations was premature in the promotion of this work."

McCullough et al. (2014)

Investigators at the American Cancer Society conducted a prospective cohort study of artificially- and sugar-sweetened soda and lymphoid cancer in 100,442 men and women from the Cancer Prevention Study-II Nutrition Cohort in the U.S. (McCullough et al., 2014). Although OEHHA's data summary noted a few significant associations with NHL in Quintiles 2 and 3, it does not describe the bottom line: this study is a negative study. The relative risk of NHL in Quintile 5, the highest exposure group, was not increased for artificially-sweetened soda (RR: 0.92; 95% CI: 0.73, 1.17) or sugar-sweetened soda (RR: 1.10; 95% CI: 0.77, 1.58). Similarly, aspartame intake was not associated with NHL risk (RR: 1.02; 95% CI: 0.84, 1.24; P-trend: 0.69, Quintile 5 vs. Quintile 1). The conclusion of the study authors is:

> "These findings do not support associations of daily consumption of artificially or sugar-sweetened carbonated beverages, or aspartame, with NHL risk."[10]

This study is reassuring because the results indicate aspartame is not associated with NHL.

Cabaniols et al. (2011)

Cabaniols et al. (2011) conducted a case-control study of brain cancer in France. Because it is a case-control study, it is not as powerful as the two prospective cohort studies described above. However, the results of this study are also reassuring. As noted in OEHHA's data summary, it showed no association between aspartame intake and brain cancer.

V. The overall evidence in epidemiologic, animal carcinogenicity, and genotoxicity studies from 1973 to the present does not warrant a high priority.

A. There is no convincing evidence that aspartame causes cancer in humans

There is no convincing evidence of carcinogenicity of aspartame in humans. The issue was first raised by two ecological studies that reported an increase in brain tumors that coincided with the introduction of aspartame in foods and beverages (Table 1). These studies, representing a classic example of the "ecological fallacy," were questioned and criticized by many scientists for a wide variety of reasons. Subsequently, three case control studies have been conducted, and no association between aspartame intake and brain cancer or other cancers was found in any of these studies (Table 1).

To date, three large prospective cohort studies of aspartame have been conducted. Two of these studies concluded that their findings do not support an association between aspartame intake and cancer (Lim et al., 2006; McCullough et al., 2014). The third study, discussed in detail earlier in this submission, reported some inconsistent associations with hematopoietic cancers in men, but not women, exposed to aspartame (Schernhammer et al., 2012). The authors concluded:

> "Although our findings preserve the possibility of a detrimental effect of a constituent of diet soda, such as aspartame, on select cancers, the consistent sex effects and occurrence of an apparent cancer risk in individuals who consume regular soda do not permit the ruling out of chance as an explanation."[11]

Schernhammer et al. (2012) also acknowledged: "our results necessarily require confirmation in other large cohorts."[12] In fact, these results have not been confirmed in two other large prospective cohorts. So, the epidemiological evidence in prospective cohort studies may be summarized as weak evidence of carcinogenicity in one prospective cohort study that was not observed in two other prospective cohort studies or three case-control studies. These findings do not warrant elevating the priority of aspartame.

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[10] McCullough et al. (2014), J Nutr doi: 10.3945/jn.114.197475, p.1.
[11] Schernhammer et al., (2012) p. 1419.
[12] Id. p. 1427.

B. There is no credible evidence that aspartame causes cancer in animal studies

There are ten carcinogenicity studies of aspartame in laboratory animals. None of these studies presents credible evidence that aspartame causes cancer in animals. The results are presented in Table 2.

1. Carcinogenicity Studies in Rats and Mice (1973-1981)

No carcinogenic effects were reported in a total of five 2-year carcinogenicity studies in rats and mice reported between 1973 and 1981. None of these five carcinogenicity studies in rats and mice found an increase in tumors attributable to aspartame. All of these studies were conducted for the purpose of supporting regulatory approval of aspartame. In the U.S., carcinogenicity studies of aspartame were submitted to and reviewed by the FDA. In addition, at FDA's request, these studies were audited by the Universities Associated for Research and Education in Pathology, Inc. In summary, these four studies provide no evidence of carcinogenicity.

2. NTP (2005) Carcinogenicity Studies in Transgenic Mice

The National Toxicology Program, a body identified by the CIC as authoritative on Proposition 65 cancer issues, conducted three carcinogenicity studies of aspartame using three different transgenic mouse models.[13] None of the NTP carcinogenicity studies is mentioned in the Background Document. The conclusion of each study was that "there was no evidence of carcinogenicity of aspartame."

The protocol was identical for each of the NTP studies. The three models used were the heterozygous p53-deficient (+/-) mouse (sensitive for spontaneous lymphomas and sarcomas), the Cdkn2a-deficient mouse (claimed to be sensitive for suspected brain carcinogens), and the Tg.AC mouse (detection of both genotoxic and nongenotoxic carcinogens and in particular sensitive for forestomach tumors). The NTP said it studied aspartame in these three transgenic or genetically manipulated mouse strains, "because this model is proposed to be susceptible to glial cell tumors of the brain."[14] The six concentration levels used were 0, 3125, 6250, 12,500, 25,000, and 50,000 ppm aspartame in NTP 2000 feed.

In all three studies, there were no tumors attributed to exposure to aspartame in either sex at any dose tested. In short, no evidence of carcinogenicity was observed in the three NTP transgenic mouse model studies with dietary levels of aspartame equivalent to 7500 mg/kg bw/day.

3. Ramazzini Institute Carcinogenicity Studies in Rats and Mice (Soffritti et al., 2005; 2007; 2010)

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[13] NTP (2005) NTP report on the toxicology studies of aspartame in genetically modified (FVB Tg.AC hemizygous) and B6.129-Cdkn2a^{tm1rdp} (N2) deficient mice and carcinogenicity studies of aspartame in genetically modified [b6.129-trp53^{tm1Brd} (N5) haploinsufficient] mice. NIH Publication No. 06-4459. October 2005, (http://ntp.niehs.nih.gov/files/GMM1_Web.pdf).

[14] NTP, Aspartame: Questions and Answers (http://ntp.niehs.nih.gov/index.cfm?objectid=03614CBD-C0A2-C207-C140B407A4043600) (last updated January 2006).

The only evidence of carcinogenicity in animals comes from unconventional and highly controversial studies conducted at the Ramazzini Institute in Bologna, Italy ("Ramazzini"). Soffritti et al. (2006, 2007, 2010) conducted three animal carcinogenicity studies of aspartame, and they reported an increased incidence of combined leukemia/lymphoma in rats and of liver and lung tumors in male mice. These studies, however, have multiple, serious problems with experimental design, interpretation of results and data reporting. Independent reviews have been highly critical of these two studies for many valid reasons. No regulatory agency has considered the Ramazzini aspartame studies to be credible evidence of carcinogenicity, and no regulatory agency has ever relied upon the Ramazzini studies of aspartame for regulatory purposes.

a. EFSA (2013) Review of the Ramazzini Studies

The European Food Safety Authority (EFSA) conducted a comprehensive review of aspartame in 2013 that included a detailed review of all three Ramazzini studies. EFSA summarized the Ramazzini studies as follows:

> "Since the last evaluation of aspartame by the [EU Scientific Committee for Food] in 2002, two long-term carcinogenicity studies in rats and one in mice were published by the European Ramazzini Foundation. The two rat studies … were considered to have methodological flaws. In addition to a high background incidence of chronic inflammatory changes in the lungs and other vital organs and tissues there is uncertainty about the diagnoses of some tumour type, which rendered the validity of the findings questionable. Moreover, EPA has recently concluded that many of the malignant neoplasms and the lymphoid dysplasias diagnosed in the studies from the European Ramazzini Foundation were hyperplasias related to unknown chronic infection in the animals not related to aspartame intake."[15]

With respect to the Ramazzini study in mice, EFSA stated:

> "The ANS Panel (EFSA ANS Panel, 2011) and EFSA (EFSA, 2011a) concluded that the hepatic and pulmonary tumour incidences reported by Soffritti et al. (2010) all fall within their own historical control ranges for spontaneous tumours. It was also noted that Swiss mice are known to have a high background incidence of spontaneous hepatic and pulmonary tumours (Prejean et al. 1973; Fox et al., 2006)." [16]
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> "Based on these data, the Panel concluded that ***the results of the studies performed by Soffritti et al. (2010) do not provide evidence for a carcinogenic effect of aspartame in mice***."[17] [emphasis added]

And finally, EFSA (2013) concluded:

> "In the hazard identification and characterization, the Panel has discussed the

[15] EFSA (2013) Opinion on the re-evaluation of aspartame (E951) as a food additive. p. 3.
[16] Id.
[17] Id.

> [Ramazzini] studies performed in mice and rats and confirmed the conclusion of previous assessments that there were major deficiencies in these studies and as such, ***they were not suitable for hazard identification*** and characterization."[18] [emphasis added]

b. FDA (2014) Evaluation of the Ramazzini Studies

On October 1, 2014, the FDA provided its opinion on all three Ramazzini studies in response to a citizen petition from K. Paul Stoller, M.D., FACHM. FDA encountered difficulty in obtaining data from Ramazzini. In response to the petition, FDA stated:

> "You contend that aspartame is a carcinogen based mainly on the results of three studies that were conducted by the Casare Maltoni Cancer Research Center of the European Ramazzini Foundation (ERF). However, FDA has not received the full data set for these studies and would need this data in order to evaluate the results and conclusions from these studies. As you mention in your petition, ERF provided to FDA only limited data and information from the ERF study published in 2006, despite FDA's request for the full set of data from ERF. Without the full data set from the study published in 2006, FDA could not conduct a complete and definitive review of this study. However, based on the available data, FDA concluded that none of the reported histopathological changes appear to be treatment related. Furthermore, the reliability and integrity of the study's results were compromised by significant shortcomings of this study, such as the presence of infection in the test animals. For these reasons, FDA determined that the data that were provided did not support ERF's conclusion that aspartame is a carcinogen. With regard to the study published in 2007, FDA has requested data from ERF but has not received any data. FDA also has not received any data for the ERF study published in 2010."[19]

c. US EPA (2012) and NTP (2011) Expert Panel Review of the Ramazzini Studies

Because of the differences of opinion between the Ramazzini scientists and independent Pathology Working Group (PWG) scientists in diagnosing leukemias and lymphomas, the U.S. EPA (2012) has decided not to rely on lymphoma or leukemia data from Ramazzini studies in Integrated Risk Information System (IRIS) assessments.[20] The PWG was sponsored by the EPA and NTP. The results of this evaluation are detailed in a publication by a group of EPA scientists (Gift et al., 2013) and in an NTP (2011) report.[21]

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[18] Id., p. 147.

[19] FDA (2014) Letter from Dr. Steven Musser, Deputy Director for Scientific Operations, Center for Food Safety and Applied Nutrition (CFSAN), FDA to Dr. K. Paul Stoller re aspartame citizen petition. Docket No. FDA-2009-P-0156. October 1, 2014.

[20] U.S. EPA (U.S. Environmental Protection Agency). 2012. Update on Ramazzini Institute Data in IRIS Assessments. https://www.epa.gov/iris/update-ramazzini-institute-data-iris-assessments

[21] Gift et al. (2013) Scientific considerations for evaluating cancer bioassays conducted by the Ramazzini Institute. Environ Health Perspect 121:1253-1263.
NTP (2011) Summary Report of the National Toxicology Program and Environmental Protection Agency Sponsored Review of Pathology Materials from Selected Ramazzini Institute Rodent Cancer Bioassays. Research Triangle Park, NC.
http://ntp.niehs.nih.gov/NTP/About_NTP/Partnerships/International/SummaryPWG_Report_RI_Bioassays.pdf

d. Schoeb et al. (2009a; 2009b) Expert Panel Review of the Ramazzini Studies

Schoeb et al., (2009a) published a letter to the editor of the Journal *Environmental and Molecular Mutagenesis* specifically addressing the diagnosis of tumors in the Ramazzini studies of aspartame and another chemical. The authors of the Letter to the Editor were: T.R. Schoeb (U. of Alabama at Birmingham), E.E. McConnell (ToxPath, Inc.), M.M. Juliana (U. of Alabama at Birmingham), J.K. Davis (Purdue U.), M.K. Davidson (FDA), and J.R. Lindsey (Emeritus, U. of Alabama at Birmingham). The authors concluded that the Ramazzini studies misclassified lesions as lymphoma that were actually lesions due to pulmonary disease.

> "Moreover, the cellular morphology shown in ERF publications for these neoplasms [Belpoggi et al., 1999; Soffritti et al., 2005] is more pleomorphic than is typical of lymphoma in rats, and the lesions appear to contain neutrophils. We believe that lesions characterized by accumulation of lymphocytes, plasma cells, and neutrophils in the lungs of conventional rats are much more likely to be due to M. pulmonis disease than to chemical induction of a rare type of lymphoma with an uncharacteristic organ distribution. Consequently, we furthermore believe that the reported induction of lymphoma by aspartame and MTBE probably is the result of exacerbation of M[ycoplasma] pulmonis disease by chemical treatment and misdiagnosis of the lesions as lymphoma."[22]

Subsequently, Schoeb et al. (2009b) published a full article on this topic, and the full publication drew the same conclusion as the letter to the editor.[23]

e. Magnuson et al. (2007) and Magnuson and Williams (2008) Expert Panel Review of the Ramazzini Studies

The two Ramazzini studies in rats were reviewed in detail by another Expert Panel, as reported by Magnuson et al. (2007). When the Expert Panel report was first written, the second Ramazzini study had not yet been published. When the second Ramazzini study was released, the Expert Panel analyzed this study in an Addendum to their report. This review of the second Ramazzini rat study was later published by Magnuson and Williams (2008) in a letter to the editor of Environmental Health Perspectives.[24] In both cases, the Expert Panel identified numerous shortcomings in the study design and conduct. The Expert Panel summarized its evaluation of the second Ramazzini study as follows:

> "considering the lack of significant differences between high dose groups and historical control cancer rates, plus the many deficits in the study design and data, it is the opinion of this expert panel that this study (Soffritti et al., 2007) fails to provide convincing evidence of aspartame carcinogenicity."[25]

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[22] Schoeb et al. (2009a) Mycoplasma pulmonis and lymphoma. Environmental and Molecular Mutagenesis. 50:1-3.
[23] Schoeb et al. (2009b) Mycoplasma pulmonis and lymphoma in bioassays in rats. Environmental Pathobiology. 46:952-959.
[24] Magnuson and Williams (2008) Carcinogenicity of aspartame in rats not proven. Environ. Health Perspect 116(6):A239-240.
[25] Magnuson et al. (2007) Aspartame: a safety evaluation based on current use levels, regulations, and toxicological and epidemiological studies. Critical Reviews in Toxicology. 37:629-727, p. 702.

Regarding the first Ramazzini study (i.e., Soffritti et al., 2005), the Expert Panel stated:

> "In summary, the Soffritti [Ramazzini] reports alleging carcinogenicity are contradicted by many publications and every scientific consideration. Many potential flaws have been suggested in this report; whether these or some other unidentified flaw is responsible for their incorrect allegations is not known. Nevertheless, it can be confidently stated that **these reports provide no credible evidence that aspartame is carcinogenic**."[26] [emphasis added]

The Expert Panel identified multiple flaws in the first study. A partial list of the defects identified by the Expert Panel includes:

- Very high incidence of infection in rats (e.g., bronchopneumonia in 81-95% of males and 69-97% of females)
- Rats were housed five per cage (high-density housing may have contributed to the high incidence of infections)
- Different groups were housed in different animal rooms (possibly accounting for differences in survival and results)
- Low survival rates at 104 weeks, which are likely due to the very high incidence of infection
- Duration of study was unconventional (rats were allowed to live until spontaneous death)
- NTP pathology review of slides confirmed the presence of autolytic tissue changes in the animals found dead
- Tumors from different tissues were inappropriately combined for analysis
- Causes of death not reported
- Most of the histopathological results were not included in the report
- No randomization of rats assigned to groups
- No information on the composition of the diet
- No adjustment of diet for the decrease in vitamins and mineral content due addition of aspartame
- Multiple problems with the statistical analyses of the data

Based on the review of the Ramazzini studies by the Expert Panel in 2007, it is evident that the Ramazzini studies in rats do not represent "scientifically valid testing." While the issue at hand is prioritization, it is interesting to note that, in order to list a chemical, it must be clearly shown through "scientifically valid testing" to cause cancer.[27]

C. Genotoxicity data does not raise the carcinogenicity concern

The genotoxic potential of aspartame has been extensively studied *in vitro* and *in vivo*, and the overwhelming weight of the scientific evidence indicates that aspartame is not genotoxic. EFSA reviewed the evidence of genotoxicity in 2013, and EFSA concluded:

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[26] Id., p. 669.
[27] Cal. Health & Safety Code §25249.8.

> "Overall, the Panel concluded that the available data do not indicate a genotoxic concern for aspartame."[28]

More recently, Kirkland and Gatehouse (2015) reviewed the *in vivo* and *in vitro* genotoxicity studies of aspartame, and they concluded:

> "There is no evidence of induction of gene mutations in a series of bacterial mutation tests. There is some evidence of induction of chromosomal damage *in vitro*, but this may be an indirect consequence of cytotoxicity. The weight of evidence from *in vivo* bone marrow micronucleus, chromosomal aberration and Comet assays is that aspartame is not genotoxic in somatic cells *in vivo*. The results of germ cell assays are difficult to evaluate considering limited data available and deviations from standard protocols. The available data therefore support the conclusions of the European Food Safety Authority (EFSA) that aspartame is not genotoxic."[29]

Thus, the weight of the scientific evidence, including many *in vitro* and *in vivo* studies, indicates that aspartame is not genotoxic. The genotoxicity data provide no basis to elevate the priority level for aspartame.

VI. Recent reviews by highly-respected regulatory and scientific organizations do not support elevating aspartame's priority.

The potential carcinogenicity of aspartame has been thoroughly reviewed by many well-respected regulatory and scientific organizations. The opinions of these organizations do not indicate aspartame's priority level should be elevated.

In response to a citizen petition alleging that aspartame is carcinogenic based on the Ramazzini studies, FDA (2014) concluded:

> "The safety of aspartame has been reviewed repeatedly, not only be FDA, but by other regulatory authorities, including those of Canada, the United Kingdom, Australia, Europe, and Japan. All these authorities agree that aspartame is safe for the general population except for individuals with phenylketonuria. Despite your many assertions, you have not identified any scientific data or other information that would cause the agency to alter its conclusions about the safety of aspartame. Therefore, FDA is denying your petition."[30]

In its extensive 263-page evaluation of aspartame, EFSA (2013) concluded:

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[28] EFSA (2013) p. 3.

[29] Kirkland and Gatehouse (2015) Aspartame: a review of genotoxicity data. Food and Chemical Toxicology. 84:161-168.

[30] FDA (2014) Letter from Dr. Steven Musser, Deputy Director for Scientific Operations, Center for Food Safety and Applied Nutrition (CFSAN), FDA to Dr. K. Paul Stoller re aspartame citizen petition. Docket No. FDA-2009-P-0156. October 1, 2014.

> "When considering all the genotoxicity, chronic toxicity and carcinogenicity studies on aspartame the Panel overall concluded that there was no convincing evidence for genotoxic or carcinogenic potential of aspartame in experimental animals."

Health Canada (2016) provides the following response to the allegation that aspartame causes cancer:

> "Allegation: Aspartame causes cancer and brain tumours
>
> Not supported.
>
> Scientists in the world-wide scientific community, including Canadian scientists, have found no link between aspartame consumption and the incidence of cancer or brain tumours from a study of the safety studies performed with aspartame."[31]

No regulatory agency in the world regulates aspartame as a carcinogen.

VII. The metabolism of aspartame does not support raising aspartame's priority level.

The OEHHA data summary notes that "aspartame is metabolized to formaldehyde, a known carcinogen."[32] However, this statement does not fully characterize the metabolism of aspartame. In fact, aspartame is completely broken down in the gastrointestinal tract by esterases and peptidases, to methanol, aspartic acid, and phenylalanine, which are all absorbed into the bloodstream.[33] Although it is true that absorbed methanol is metabolized by stepwise oxidation to formaldehyde and ultimately formic acid, this conversion is so efficient that you cannot measure formaldehyde in the blood.[34] The half-life of the formaldehyde metabolite in blood is reported to be about one minute.[35] EFSA (2013) stated that the carcinogenicity of formaldehyde is only observed under occupational exposure by inhalation and that oral exposure is not linked to cancer in animals.[36] EFSA (2013) concluded that "based on recent measurements of basal levels of formaldehyde in blood and on the modelling of its biological turnover and steady state concentration in cells, formaldehyde formed from aspartame-derived methanol was not of safety concern at the current exposure estimates or at the ADI of 40 mg/kg bw/day."[37] In addition, EFSA (2013) concluded that "there is no safety concern from the levels of methanol released from aspartame under the current uses and permitted use levels."[38]

FDA reached a similar conclusion in 2014:

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[31] Health Canada (2016) http://hc-sc.gc.ca/fn-an/securit/addit/sweeten-edulcor/aspartame-eng.php
[32] OEHHA data summary of aspartame (2016) p. 5.
[33] Magnuson BA et al. (2016) Biological fate of low-calorie sweeteners. Nutrition Reviews. 74(11):670-689.
[34] EFSA (2013) p. 4.
[35] Id., p. 110.
[36] Id., p. 126.
[37] Id., p. 5.
[38] Id., p. 126.

> "In the case of methanol from the consumption of aspartame, FDA assessed its safety from the results of toxicological testing on aspartame itself and from the estimated exposure to methanol resulting from the use of aspartame in food. Although aspartame ingestion results in the production of methanol, the levels formed from consumption of aspartame are small compared to that from consumption of other foods (e.g. apples or pears). FDA determined these levels to not be of toxicological concern, and is not aware of any information to the contrary."[39]

Further, there is no safety concern regarding the metabolite formic acid, which is excreted in urine or converted to carbon dioxide.[40] Thus, the data on the metabolism of aspartame, which has been known for decades, does not support raising aspartame's priority level.

VIII. Conclusion

In conclusion, aspartame's priority level should not be raised from its current level of "at the bottom of the medium category" as established by the CIC in 2009. The one new additional animal carcinogenicity study and the additional epidemiologic studies, which were not considered by the CIC in 2009, do not raise the level of concern for aspartame.

[39] FDA (2014) Letter from Steven Musser, Ph.D., Deputy Director for Scientific Operations, Center for Food Safety and Applied Nutrition (CFSAN), FDA to Mrs. Betty L. Martini. Citizen Petition Denial. Docket No. FDA-2002-P-0247. Oct. 1, 2014, p. 3.
[40] EFSA (2013) p. 4.