



May 15, 2014

The Center for Science in the Public Interest (CSPI) thanks the 2015 Dietary Guidelines Advisory Committee (DGAC) for the opportunity to submit comments on the **Subcommittee (SC) request 5-1: Food safety.**

CSPI is a non-profit consumer education and advocacy organization that since 1971 has been working to improve the public's health through better nutrition and food safety policies. CSPI's work is supported primarily by its 900,000 subscribers to its *Nutrition Action Healthletter*, the nation's largest-circulation health newsletter. CSPI is an independent organization that does not accept any government or corporate funding.

We respectfully submit the following comments on the **safety of aspartame** to the DGAC.

We would be pleased to provide more information to the DGAC upon request. Please contact Lisa Lefferts, MSPH at 202-777-8317 or [llefferts@cspinet.org](mailto:llefferts@cspinet.org).

## Aspartame

### Summary

We applaud the DGAC for considering the safety of aspartame. Aspartame is used in thousands of products and is one of the most widely consumed artificial sweeteners in the world.

New research has been published that calls into question the safety of aspartame. A commentary published earlier this year summarizing and analyzing the cancer data calls the need for a re-evaluation “an urgent matter of public health.”<sup>1</sup>

Unfortunately, a recent re-evaluation by the European Food Safety Authority (EFSA) glossed over some key studies and omitted others relevant to evaluating the potential carcinogenicity of aspartame. The EFSA re-evaluation was criticized for conflicts of interest<sup>2</sup> and for cutting and pasting sections of an industry review into a draft of the report.<sup>3</sup> An independent, objective re-evaluation is urgently needed.

There is compelling evidence of harm. Three rodent bioassays<sup>4</sup> in two species, both genders, found aspartame to cause cancer at multiple sites. These studies were published in peer-reviewed journals, two in *Environmental Health Perspectives*, published by the U.S. National Institute of Environmental Health Sciences (NIEHS). The finding in a recent prospective human cohort study<sup>5</sup> of a slight but statistically significant increased risk in the incidence of similar (lymphohematopoietic) tumor types as seen in two animal studies lends further support to the conclusion that aspartame is likely carcinogenic in humans.

The three rodent bioassays were conducted by an independent laboratory – the Ramazzini Institute (RI), one of the largest and longest-running bioassay programs in the world. The RI and RI methodology have come under sharp criticism from multiple sources, including EFSA and industry.

The Center for Science in the Public Interest (CSPI), with help from qualified experts in evaluating cancer bioassays, carefully investigated that criticism and the cancer evidence on aspartame. CSPI has a longstanding commitment to addressing Americans’ overconsumption of refined sugars, and thus we support the development and use of safe non-caloric sweeteners. CSPI was keen to determine if the RI produced reliable, credible results and if its rodent studies, particularly the studies finding evidence of aspartame’s carcinogenicity, were valid.

Below we summarize our investigation and conclusions, including the experts and sources we consulted—and which we recommend that the Committee consult. In short, we found

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<sup>1</sup> Leukemias and lymphomas (seen in the rodent studies) and non-Hodgkins lymphoma and multiple myeloma, and possibly leukemia, in the human study.

the criticism to be largely without merit, and that there was convincing evidence that aspartame is likely to cause cancer in humans.

### **CSPI's Investigation into the Cancer Evidence for Aspartame**

We spoke with many scientists in the course of our investigation but in particular consulted three experts:

- Kathleen Burns, PhD, Director, Sciencecorps; Dr. Burns has twenty-five years of experience with state and federal agencies in toxicology and public health and is the founder of Sciencecorps.
- James Huff, PhD, Guest Researcher, NIEHS Sciences (acting as an individual and not as a representative of the NIEHS); Dr. Huff, now retired, is the former chief of the International Agency for Research on Cancer (IARC) Monographs and led the US National Toxicology Program bioassay program.
- Ronald Melnick, PhD, of Ron Melnick Consulting LLC; Dr. Melnick is a retired NIEHS Senior Toxicologist and a frequent IARC panelist.

Reviews by the International Agency for Research on Cancer (IARC) are widely regarded as the gold standard to determine whether or not chemicals or other agents could lead to cancer. In addition to our experts with IARC affiliations, we frequently sought guidance from texts published by IARC.

CSPI Senior Scientist Lisa Lefferts, MSPH, conducted the investigation, and Michael Jacobson, PhD, Executive Director of CSPI, oversaw it.

We would be pleased to present the findings of our investigation in more detail and provide any other information that might be helpful to the committee. The committee may wish to consult directly with the above-named (or other) experts.

In the course of our investigation we endeavored to review all relevant published articles and reports relating to RI methodology and the interpretation of RI findings, in addition to animal and epidemiological studies relevant to aspartame carcinogenicity. We found several sources, all by scientists who work for U.S. government agencies or were working on behalf of government agencies, to be particularly helpful:

- The 2011 Summary Report of the National Toxicology Program/Environmental Protection Agency (NTP/EPA)-sponsored review of pathology materials from five selected RI bioassays.<sup>6</sup>
- The 2011 Individual Pathology QA (Quality Assessment) Review and Pathology Working Group (PWG) Coordinator's Reports for RI Studies<sup>7</sup> on each of the five

chemicals in the Summary Report of the NTP/EPA-sponsored review cited above, in particular, those for methyl-tertiary-butyl ether (MTBE) and methanol since, like aspartame, they are metabolized to formaldehyde, a recognized carcinogen.

- The 2004 NIEHS PWG Chairperson's Report on the first RI cancer bioassay of aspartame.<sup>8</sup>
- The 2013 paper by Gift *et al.*, "Scientific considerations for evaluating cancer bioassays conducted by the Ramazzini Institute."<sup>9</sup>
- The 2008 Caldwell *et al.* paper, "Evaluation of evidence for infection as a mode of action for induction of rat lymphoma,"<sup>10</sup> and the 2009 response by Caldwell *et al.* to letters to the Editor.<sup>11</sup>
- The 2002 paper by Huff comparing chemicals studied and evaluated in long-term carcinogenesis bioassays by both the Ramazzini Foundation and the National Toxicology Program.<sup>12</sup>

Four of those six sources were not considered by EFSA in its re-evaluation of aspartame.<sup>ii</sup> In addition, the recent re-analysis of the industry studies of aspartame<sup>1</sup> was published after the EFSA re-evaluation.

### **Key Conclusion: Aspartame is a likely carcinogen in humans.**

Based on our investigation, we concluded that aspartame causes cancer in animals, and thus is likely to cause cancer in humans.

The three RI bioassays of aspartame that found significant associations between aspartame treatment and multiple tumor types in two rodent species provide ample evidence that aspartame is carcinogenic in animals. IARC defines "sufficient evidence of carcinogenicity" in experimental animals as follows:

*Sufficient evidence of carcinogenicity:* The Working Group considers that a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols.<sup>13</sup>

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<sup>ii</sup> Only the Summary Report dated November 29, 2011, is cited in the EFSA re-evaluation (European Food Safety Authority (EFSA), 2013. [Scientific Opinion on the re-evaluation of aspartame \(E 951\) as a food additive](#). EFSA Journal 11(12): 3496.) The article by Gift *et al.* was considered after the public consultation had ended and the final re-evaluation was written (EFSA, 2013. [Statement on two reports published after the closing date of the public consultation of the draft Scientific Opinion on the re-evaluation of aspartame \(E951\) as a food additive](#). EFSA Journal 11(12):3504 (10 pp).)

Consequently, it is appropriate to consider aspartame to have carcinogenic potential in humans. That is supported by an international scientific consensus as reflected by IARC and is assumed (unless there is persuasive evidence for a contrary view) by U.S. government regulatory agencies:

... it is biologically plausible that agents for which there is sufficient evidence of carcinogenicity in experimental animals ... also present a carcinogenic hazard to humans. Accordingly, in the absence of additional scientific information, these agents are considered to pose a carcinogenic hazard to humans.<sup>13</sup>

The finding in a recent prospective cohort study<sup>5</sup> of a slight but significant increased risk of similar (lymphohematopoietic) tumor types as seen in two animal studies lends further support to the conclusion that aspartame is likely carcinogenic in humans.

### **Aspartame caused cancers at multiple sites in three independent animal bioassays.**

While lymphomas/leukemias (L/L) were the most common tumors seen in RI's bioassays, aspartame treatment was also associated with tumors at other sites: transitional-cell carcinomas of renal pelvis/ureter in female rats, malignant schwannomas in male rats, mammary cancers in female rats whose exposure began in utero, and liver and lung tumors in male mice when exposure began in utero. The fact that aspartame causes cancer at multiple sites in animals adds to the weight of evidence that aspartame poses a cancer risk to humans. Differences in study design (numbers of animals; whether including in utero exposure or not) between the two RI rat studies likely account for differences in tumor sites observed.

- Transitional-cell carcinomas of renal pelvis/ureter are *highly significant* and *extremely rare* in rats. In 17 studies using 2,669 control Sprague-Dawley rats, those tumors were found in only 1 male and 1 female, and in 10 studies using 1,060 control Fischer 344 rats, they were found in only 1 male.<sup>14</sup> In the first RI bioassay of aspartame, transitional-cell carcinomas were found in 21/1500 aspartame treated rats, versus *none* in 300 controls. The carcinomas in females showed a positive trend ( $p < 0.05$ ), and there was a significant increase ( $p < 0.05$ ) in high-dose females. Furthermore, statistically significant increases of dysplastic lesions plus carcinomas of the renal pelvis/ureter were seen in the four top doses, with a positive trend in females ( $p < 0.01$ ). IARC states:

The occurrence of lesions presumed to be preneoplastic may in certain instances aid in assessing the biological plausibility of any neoplastic response observed.<sup>13</sup>

- Lymphomas and leukemias were observed in both of the RI rat studies. In the first RI study, there was a positive significant trend in males and females and a

significant increase in females at five doses, compared to controls. In the second study, there was a significant dose-related increase in females, especially at the high dose ( $p < 0.01$ ) and in high dose males.

- Although L/L diagnoses by RI pathologists have been criticized (see discussion below), the only NTP-sponsored review that focused specifically on an RI study of aspartame (the 2004 PWG Report of the first RI study) states, “The diagnoses of lymphatic and histocytic neoplasms in the cases reviewed were generally confirmed.”
- Malignant schwannomas of peripheral nerves showed a positive trend in male rats ( $p < 0.05$ ) in the first RI bioassay of aspartame. The 2004 PWG report on aspartame states:

cases diagnosed as malignant schwannoma of the cranial nerve were generally confirmed by the PWG, although some members “preferred a diagnosis of sarcoma, NOS.”

- Mammary cancers showed a significant dose-related increase in female rats ( $p < 0.05$ ), and there was a significant increase in high dose females ( $p < 0.05$ ), in the second RI bioassay of aspartame.
- Hepatocellular carcinomas in male mice showed a significant dose-related increased trend ( $p < 0.01$ ), and a significant increase in the highest dose ( $p < 0.01$ ) and next highest dose ( $p < 0.05$ ) compared to controls.
- Alveolar-bronchiolar carcinomas in male mice showed a significant dose-related increased trend ( $p < 0.05$ ) and a significant increase at the highest dose ( $p < 0.05$ ) compared to controls.

EFSA dismissed all those cancer findings.<sup>15</sup> It referred to “methodological concerns” and contended that the RI studies were “flawed” due to a high background incidence of chronic inflammatory changes in the lungs and other vital organs and tissues and the uncertainty regarding the correctness of the diagnoses of some tumor types.<sup>15</sup> Since the RI uses lifetime studies, chronic inflammatory changes are not unexpected and do not invalidate the results. We discuss below the “uncertainty regarding the correctness of the diagnoses of some tumour types,” and conclude that the argument has little merit.

Disappointingly, EFSA ignored or minimized “methodological concerns” (discussed below) with studies that found *no* adverse effects of aspartame.

For the kidney tumors, EFSA argued that high doses of irritant chemicals produce renal pelvic calcification as a result of imbalances in calcium metabolism that are specific to the

rat and of no relevance for humans.<sup>16</sup> However, that reasoning is not persuasive. For example, pre-neoplastic changes were seen at low doses. Furthermore, chemical-induced, rarely occurring kidney tumors are considered clear evidence of carcinogenicity, according to the experts we consulted.

EFSA dismissed the mammary carcinomas, saying they were “not considered indicative of a carcinogenic potential of aspartame since the incidence of mammary tumours in female rats is rather high and varies considerably between carcinogenicity studies.” Similarly, EFSA dismissed the hepatic and pulmonary tumors in mice by concluding that they fell within the historical control range<sup>15</sup> for spontaneous tumors, and that spontaneous tumors in control mice generally show wide variations in incidence.<sup>16</sup> That contradicts recommendations from the International Agency for Research on Cancer (IARC), which states:

It is generally not appropriate to discount a tumour response that is significantly increased compared with concurrent controls by arguing that it falls within the range of historical controls ....<sup>13</sup>

**Studies that find links between aspartame and cancer outweigh those that did not. The protocols used by the RI provide advantages for identification of chemical-related neoplasia that are not obtained from other bioassays.**

In 2007, FDA commented on the first RI bioassay,<sup>17</sup> stating:

Considering results from the large number of studies on aspartame's safety, including five previously conducted negative chronic carcinogenicity studies, a recently reported large epidemiology study with negative associations between the use of aspartame and the occurrence of tumors, and negative findings from a series of three transgenic mouse assays, FDA finds no reason to alter its previous conclusion that aspartame is safe as a general purpose sweetener in food.

However, previous studies that found no evidence of cancer had important limitations and are trumped by the more recent positive studies. Specifically:

- The five industry-sponsored negative chronic carcinogenicity studies reporting no evidence of carcinogenicity in rodents were too small (36-40 animals/sex/dose, whereas at least 50 animals/sex/dose is recommended<sup>iii</sup>) and thus lacked sensitivity and statistical power. Three of those old studies were never published in peer-reviewed scientific literature and were only recently made more widely available to the public on the EFSA website. A recent analysis<sup>1</sup> noted other

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<sup>iii</sup> US Food and Drug Administration, 2006. [Toxicological Principles for the Safety Assessment of Food Ingredients: Redbook 2000. IV.C.6 Carcinogenicity Studies with Rodents](#) states, “It is recommended that carcinogenicity studies begin with at least 50 animals per sex per group.”

important limitations (e.g., statistically significant decreases in feed consumption, body weight, and survival may have limited the full expression of carcinogenic effects).

- The negative findings from a series of three transgenic mouse assays are not compelling. NTP no longer considers such studies reliable for cancer evaluation screening. According to NTP, “there is uncertainty whether the study possessed sufficient sensitivity to detect a carcinogenic effect.”<sup>18</sup>
- The epidemiology study by Lim *et al.* had many subjects, but suffered from major limitations. For example, aspartame was not approved until the subjects were in their late 30s to 50s or older, the study had poor data on consumption levels, and few of the subjects drank large amounts of diet soda. The study was worthless for detecting effects of exposures beginning early in life followed by life-long consumption. It is unlikely that the Lim study could have detected any carcinogenic potential of aspartame.

Furthermore, since the FDA’s 2007 statement about the first RI study, RI has published two additional animal bioassays of aspartame that also found evidence of carcinogenicity.

In contrast to the negative studies, the positive studies are more robust. First, they are larger. The first (2006) RI bioassay used 100-150 animals/sex/group, for a total of 1,800 animals (compare to the total of 280-440 animals/study in the industry studies). That study was far larger than any other bioassay of aspartame. That confers an important advantage in being able to detect rare tumors. The second bioassay used 70 animals/sex/treatment group, and the third used 62-122/sex/group—both far more animals than were used in industry’s old studies.

Second, two of the RI studies included gestational exposure, which is particularly important in light of the widespread recognition of greater susceptibility to carcinogens in early life.

Third, all of the RI studies used a lifetime protocol, instead of the 2-year protocol typically used by NTP and others. Two years for a rodent is roughly equivalent to retirement age in humans. The two-year design was originally developed for use in identifying occupational carcinogens. A lifetime protocol has the advantage of being able to detect cancers that occur later in life (after retirement age) and is more relevant for consumer (vs. occupational) exposures.<sup>19</sup>

Gift *et al.* reviewed the body of evidence and concluded that the RI methodology yielded several discrete advantages:

Although the protocols characteristic of RI studies can cause interpretive challenges, aspects of the RI design, including gestational exposure, lifespan observation, and

larger numbers of animals and dose groups, may impart advantages that provide chemical risk assessors with valuable insights for the identification [of] chemical-related neoplasia not obtained from other bioassays.

In addition to the RI animal studies, the most comprehensive long-term epidemiologic study<sup>5</sup> to evaluate the association between aspartame and cancer risk was published since the 2007 FDA statement. That prospective study reported a significant positive association between diet soda intake ( $\geq 1$  serving/day) and risks of non-Hodgkin's lymphoma (NHL) (RR: 1.31; 95% CI: 1.01, 1.72) and multiple myeloma (MM) (RR: 2.02; 95% CI: 1.20, 3.40) in men, compared with men who did not consume diet soda. None of the analyses showed a significant association among women.

There was also an increased risk of leukemia with a high intake of diet soft drinks compared to a low intake in the combined cohort of men and women (RR: 1.42; 95% CI: 1.00, 2.02). Intake of aspartame was directly associated with the increased risk of NHL and multiple myeloma and possibly leukemia in men. It is noteworthy that lymphohematopoietic tumors have been linked to aspartame intake in both humans and animals.

### **Mechanism of Action**

Several researchers<sup>1,5,9</sup> have noted that aspartame metabolizes to formaldehyde. Formaldehyde has been classified as a human carcinogen, and, like aspartame, causes lymphohematopoietic and other cancers in animals. (The evidence that formaldehyde causes those cancers in humans came long after the studies in animals.) Studies performed by the RI laboratory *as well as other laboratories* corroborate that other chemicals that metabolize to formaldehyde, namely MTBE and methanol, cause cancer.

This mechanism of action is consistent with the available human data on aspartame risk. The authors of the most comprehensive long-term epidemiologic study to evaluate the association between aspartame and cancer risk<sup>5</sup> hypothesized that the sex differences they observed in lymphohematopoietic cancers (i.e., the increased risk in men for non-Hodgkin's lymphoma and multiple myeloma was not observed in women) might have been due to the recognized higher enzymatic activity of alcohol dehydrogenase type I (ADH1) in men, which possibly induced higher conversion rates from methanol to formaldehyde.

Furthermore, since concurrent alcohol (ethanol) consumption inhibits methanol metabolism, they stratified the results in men by alcohol intake. They assumed that men with lower regular alcohol consumption would have higher formaldehyde conversion rates if they consumed large amounts of diet soda, and consequently, higher cancer risk. In fact, this was the case. Risks of non-Hodgkin's lymphoma, multiple myeloma, and leukemia were higher in men with a lower alcohol intake. Similarly, Soffritti notes that the differing results between male and female rats exposed to aspartame (more lymphohematopoietic

cancers observed in females vs. males) may be due to the higher activity of ADH1 in female rats than in male rats.<sup>1</sup>

### **Criticisms of the RI and its research**

Industry and some government agencies have criticized the RI and its studies on several grounds. The criticism does not stand up to careful scientific scrutiny. Instead, there is compelling evidence that:

1. The RI produces reliable results largely consistent with those of NTP and other laboratories.
2. Only the *numerical magnitude* of lymphomas/leukemias (L/L) in RI rats diagnosed by different pathologists has varied; the presence of lymphomas/leukemias in RI treated animals is not in dispute. The L/L observed are chemical specific and not induced by infection, as was previously hypothesized by EFSA.

Below we summarize the basis for each of these conclusions.

#### **1. The RI produces reliable results largely consistent with those of NTP and other laboratories.**

A review of chemicals evaluated in long-term cancer bioassays by both the U.S. NTP and the RI, “the two largest, longest existing, and most well-established bioassay programs in the world,” found remarkably consistent results.<sup>19</sup>

The 2011 Summary Report of the EPA/NTP-sponsored review of the RI declared the RI to be “a well-organized, clean facility,” where staff “apply meticulous detail to the necropsy and to the recording, collecting, and archiving of materials and tissues.” The 2011 EPA/NTP-sponsored Pathology QA Reviews and PWG Coordinator’s Reports documented that all slides required were present, histologic quality of the sections were considered “very good” by the QA pathologist, “with no deficiencies that interfered with the examination or the interpretation of histopathologic changes that were present,” and “neither the occasional cases with tissue autolysis nor the use of alcohol fixation presented diagnostic difficulties.”

We note those conclusions since they contradict criticism of the RI.

Based on the NTP-EPA sponsored review, where there was good agreement between RI and PWG pathologists in diagnosing solid tumors, the EPA decided to continue to consider RI solid tumor data in its assessments.<sup>20</sup>

Similarly, Gift *et al.* concluded that “RI bioassay results for cancer endpoints other than respiratory tract lymphoma/leukemia, and inner ear and cranium neoplasms, are generally

consistent with those of NTP and other laboratories.” For respiratory tract L/L, Gift *et al* note that diagnoses may vary and depend on pathologists’ judgments, process of review, and criteria for a diagnosis. As a result, EPA has decided not to rely on L/L data in its Integrated Risk Information System (IRIS) assessments. This issue is examined in more depth, below.

**2. Only the *numerical magnitude* of lymphomas/leukemias (L/L) in RI rats diagnosed by different pathologists has varied; the presence of lymphomas/leukemias in RI treated animals is not in dispute. The L/L observed in RI rats are chemical specific and not induced by infection, as was previously hypothesized by EFSA.**

We will examine these two statements separately

**2A. Only the *numerical magnitude* of L/L in RI rats diagnosed by different pathologists has varied; the presence of L/L is not in dispute.**

Concerning the EPA-NTP sponsored review of RI studies, the EPA states:

The report pointed out some instances where the presence of respiratory infections in RI study animals made definitive diagnoses difficult, and that some RI diagnoses, primarily certain leukemias and lymphomas, were not considered to be malignant tumors. As a result, PWG scientists found fewer numbers of leukemias and lymphomas than had been originally reported by the RI. Because of the *differences of opinion* [emphasis added] between the RI and PWG scientists in diagnosing leukemias and lymphomas, EPA has decided not to rely on data from the RI on lymphomas and leukemias in IRIS assessments.

As part of the EPA-NTP sponsored review of RI studies, a pathologist (the QA pathologist, under contract to NTP) examined all the tissues, and then a group of pathologists (the pathology working group, or PWG, coordinated by a pathologist under contract to NTP and composed of pathologists from FDA, Charles River Labs, Leicester University in the UK, and Biotechnics, a private firm) examined a subset of tissues.

The 2011 PWG reports, the QA pathologist reviews, and the RI bioassays provide three sets of data on L/L and other tumors in RI rats. For the RI study on MTBE, only “a few” of the original RI diagnoses of lymphoma/leukemia were *not* confirmed by the QA pathologist.

In other words, these two datasets (the L/L diagnosed by the QA pathologist and by the RI pathologist) are in close agreement. The PWG found lymphomas in female rats, although fewer than RI or QA pathologists. *All three datasets agree with the diagnosis of L/L; only the exact magnitude of the response is at issue.*

While it is not known which dataset is the most accurate, the article by Gift *et al.* suggests several reasons why the PWG dataset might differ from the QA and RI pathologists. Gift *et al.* stated, “in some cases, the PWG panel reviewed lung lymphomas without also reviewing potentially corroborating diagnoses in other tissues made by QA pathologists.”

Furthermore, the PWG panel “typically based lymphoma/leukemia conclusions on the occurrence of the lesions outside the lung (e.g., thymus, spleen, liver, lymph nodes).” In effect, they could not find what they failed to look for. As Gift *et al.* conclude, “The limited number of slides reviewed by the 2011 PWG panel affected the ability to fulfill the requirement of additional sites for a definitive diagnosis.” That would of course limit the number of cancers that could be found to those that had metastasized to other tissues—an advanced stage that should not be required for diagnosis. These practices would logically explain why the QA and Ramazzini Institute pathologists, who examined all tissues, diagnosed more lymphomas/leukemia than the PWG.

As mentioned previously, an earlier (2004) PWG report that focused *specifically* on the first RI bioassay of aspartame states “The diagnoses of lymphatic and histiocytic neoplasms in the cases reviewed were generally confirmed.”

Differences in opinions on the magnitude of a cancer response create uncertainties for quantitative risk assessment. However, such differences do not create uncertainty in identifying that aspartame poses a carcinogenic hazard.

## **2B. L/L in RI rats is due to chemical exposure, not infection.**

The Caldwell *et al.* (2008) and Gift *et al.* (2013) analyses note a number of reasons why the L/L observed in RI rats are chemical-specific and not induced by infection, as hypothesized by EFSA.

For example, respiratory infections (e.g., by *Mycoplasma pulmonis*) are frequent in aging rats, and RI rat bioassays are “lifetime” studies (unlike NTP and other bioassays that are terminated at two years—roughly equivalent to retirement age in humans), but L/L are only reported for a few RI rat bioassays. Of over 200 bioassays by the RI, dose-related increases of L/L have only been reported in 10, according to Gift *et al.* Thus the link between respiratory infections such as those common in aging rats and L/L is unconvincing.

Also, Caldwell cites bioassays of propylene oxide and ethylene oxide in F344 rats that found *M. pulmonis* infection not related to chemical exposure, but which affected survival, and yet there was no increase in L/L, suggesting that infection did not cause the tumors.

Gift *et al.* disagreed with EFSA, stating that “The diagnosis of increased lymphomas/leukemias in a minority of RI studies (i.e., ca. 5%) and consistency of

diagnoses between RI and non-RI studies for some chemicals (*especially those metabolized to formaldehyde*) suggests that a regular misassociation of the endpoint and chemical exposures has not occurred in RI studies" [emphasis added].

It is noteworthy that even the PWG found a dose-related increase in L/L for MTBE, even though they diagnosed fewer L/L than RI pathologists or the QA pathologist.

Furthermore, in the first RI rat bioassay of aspartame, a positive significant trend in L/L was observed in both males and females, with significant increases in L/L in females at five doses. In the second RI rat bioassay, there was a significant dose-response increase in L/L in females, especially at the high dose ( $p < 0.01$ ) and in high-dose males.

While the Gift *et al.* article discusses several factors that may complicate interpretation of RI findings, it would be incorrect to conclude, as EFSA has, that lymphomas/leukemias in RI bioassays are not aspartame-related. As noted above, there are several possible reasons for the differences between RI and PWG diagnoses of lymphoma/leukemia and those are only differences of degree.

All the same, it is the case that light microscopy is not the best technique for diagnosing and quantifying L/L. Gift *et al.* note that the 2011 PWG review of RI studies showed that, for lymphomas and leukemias, pathologic determinations using light microscopy are problematic, especially when confounded by infiltrates from an infectious disease. Such diagnosis may vary and depend on pathologists' judgments, process of review, and criteria for a diagnosis.

Gift *et al.* conclude "A causal association between respiratory infections and lymphomas is less likely than the possibility that RI study results have been *misinterpreted* [emphasis added] due to confounding end-of-life respiratory infections.

Gift *et al.* include a discussion on other techniques, such as polymerase chain reaction (PCR) and microdissection assays, that can be used to more definitively diagnose L/L. Currently, a collaborative effort between NTP and the RI is underway to better characterize L/L in RI rats.<sup>1</sup>

## **Recommendations to the DGAC**

We urge the DGAC to:

- Acknowledge that there are legitimate reasons to doubt the safety of aspartame, that there is not a "reasonable certainty of no harm" from aspartame, and that the evidence linking aspartame and cancer is compelling.

- Recommend that the National Toxicology Program promptly conduct the appropriate analyses of available studies to resolve any lingering uncertainties in the animal data on cancer (e.g., by using PCR analyses or other diagnostic techniques to better characterize the magnitude of the lymphoma/leukemia response in RI rats).
- Advise consumers, particularly pregnant women and children, to avoid aspartame, in light of evidence of carcinogenicity from lifetime animal studies, including those beginning exposure *in utero*.
- Recommend that the FDA reconsider its position on aspartame and determine whether the status of aspartame as an approved food additive should be revoked or modified.

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#### Endnotes

<sup>1</sup> Soffritti M, Padovani M, Tibaldi E, et al. The carcinogenic effects of aspartame: The urgent need for regulatory re-evaluation. *Am J Ind Med.* 2014; 57:383-97.

<sup>2</sup> Millstone E. EFSA on Aspartame. December 16, 2013. Available at <https://www.sussex.ac.uk/webteam/gateway/file.php?name=millstone-on-efsa-on-aspartame-16dec2013.pdf&site=25>. Accessed April 29, 2014. Submitted to EFSA as part of the public consultation on the draft EFSA scientific opinion on the re-evaluation of aspartame as a food additive.

<sup>3</sup> Cicolella A. Re-evaluation of Aspartame. April 9, 2013. Available at <http://www.efsa.europa.eu/en/events/documents/130409-p08.pdf>. Accessed April 29, 2014. Submitted to EFSA as part of the public consultation on the draft EFSA scientific opinion on the re-evaluation of aspartame as a food additive.

<sup>4</sup> (a) Soffritti M, Belpoggi F, Degli Esposti D, et al. First experimental demonstration of the multipotential carcinogenic effects of aspartame administered in the feed to Sprague-Dawley rats. *Environ Health Perspect.* 2006; 114:379-85.

(b) Soffritti M, Belpoggi F, Tibaldi E, et al. Lifespan exposure to low doses of aspartame beginning during prenatal life increases cancer effects in rats. *Environ Health Perspect.* 2007; 115:1293-7.

(c) Soffritti M, Belpoggi F, Manservigi M, et al. Aspartame administered in feed, beginning prenatally through life span, induces cancers of the liver and lung in male Swiss mice. *Am J Ind Med.* 2010; 53:1197-206.

<sup>5</sup> Schernhammer ES, Bertrand KA, Birmann BM, et al. Consumption of artificial sweetener- and sugar-containing soda and risk of lymphoma and leukemia in men and women. *Am J Clin Nutr.* 2012; 96:1419-28.

<sup>6</sup> National Toxicology Program and U.S. Environmental Protection Agency. Summary Report of the National Toxicology Program and Environmental Protection Agency-Sponsored Review of Pathology Materials from Selected Ramazzini Institute Rodent Cancer Bioassays. November 29, 2011. Available at

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<sup>7</sup> Pathology Working Group (PWG) Reports for Ramazzini Institute Studies: Pathology QA (Quality Assurance) Review and PWG Coordinator's Report for Ramazzini Institute Acrylonitrile Studies; for Ethyl-tertiary-butyl Ether; for Vinyl Chloride; for Methyl-tertiary-butyl Ether Studies; and Methyl Alcohol Studies. Available at <http://www.nih.gov/icd/od/foia/index.htm>. Accessed April 29, 2014.

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