Evaluation of Ramazzini Institute Aspartame Studies – and EFSA's Assessment

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Who is CSPI and What is Our Agenda?

- CSPI is a leading US science-based consumer NGO
- Our work on safety of nitrite, nitrate, sulfites, olestra, Violet 1 led to US FDA action
- CSPI is extremely active in efforts to reduce sugar consumption. We applaud the use of safe low/no calorie sweeteners
- I'm a Senior Scientist at CSPI, public health, toxicology background, have worked for NGOs for the past 25 years, never worked for industry.
- Our comments are at http://cspinet.org/new/pdf/aspartame-efsa-final-comments-21913.pdf

CSPI Comments to EFSA: Co-signers

- Kathleen Burns, PhD, Director, Sciencecorps
  - 25 years for state and federal agencies in toxicology, public health before founding Sciencecorps
- James Huff, PhD, Guest Researcher, National Institute of Environmental Health Sciences
  - Former chief of IARC Monographs
  - Led NTP Bioassay program
- Ronald Melnick, PhD, Ron Melnick Consulting
  - Frequent IARC Panelist, Retired NIEHS Sr. Toxicologist

Is the Ramazzini Institute a Credible, Professional Organization?

Rumors abound, but what is the evidence?

The Pathology Working Group reports for ERF Studies: http://www.nih.gov/icd/od/foia/index.htm which were not considered by the EFSA Panel add important detail to the Summary Report of the NTP-EPA-Sponsored Review (Nov. 29, 2011), together comprising the most comprehensive review of ERF laboratory practices and pathology evaluations available

- "well-organized, clean facility"
- "apply meticulous detail to the necropsy and to the recording, collecting, and archiving of materials and tissues."
- "The SOPs, GLP documents, and necropsy records were within GLP expectations."
- All slides required were present

Ramazzini: What is the Evidence? (Continued)

- Histologic quality of the sections “very good” said QA pathologist, with “no deficiencies that interfered with the examination or the interpretation of histopathologic changes that were present”
- “neither the occasional cases with tissue autolysis nor the use of alcohol fixation presented diagnostic difficulties”

Is the Ramazzini Institute a Credible, Professional Organization?

- “The two largest, longest-existing, and most well-established bioassay programs in the world are the Ramazzini Foundation and the National Toxicology Program”
- a comparative review found remarkably consistent results.

(Source: Huff, Ann NY Acad Sci 2002 Dec;982:208-30)

Are Ramazzini Institute Tumor Diagnoses Reliable?

- QA pathologists of the PWG and the PWG itself agreed with diagnoses made by RI pathologists, except for the *numerical magnitude* of lymphoma responses
  - For MTBE, only "a few" of the original diagnoses of lymphoma/leukemia were *not* confirmed by the QA pathologist. The PWG found lymphomas in female rats, although fewer than RI or QA pathologists.
  - Who is right?

Are Ramazzini Institute Tumor Diagnoses Reliable?

- A 2004 PWG Report of the RI study on aspartame states “The diagnoses of lymphatic and histocytic neoplasms in the cases reviewed were generally confirmed.”
- Diagnoses of cancers other than lymphomas/leukemias *are not at issue*
  - PWG QA pathologists largely agreed with diagnoses made by ERF pathologists, except for some lymphomas/leukemias
  - US EPA will continue to use RI solid-tumor data

Does Infection, Not Aspartame, Explain Lymphomas/Leukemias?

- The EFSA Panel did not consider these two key sources which specifically evaluate the EFSA hypothesis that infection in rats, not aspartame, explain lymphomas/leukemias

  - J. C. Caldwell et al, "Evaluation of Evidence for Infection as a Mode of Action for Induction of Rat Lymphoma," *Env. & Molecular Mutagenesis 49*: 155-164, 2008
  - J. C. Caldwell et al, "Response to Letters to the Editor: Caldwell et al. [2008]," *Env. & Molecular Mutagenesis 50*:6-9, 2009

Does Infection, Not Aspartame, Explain Lymphomas/Leukemias?

- The arguments by Caldwell et al are compelling. For example:
  - Studies of ethylene and propylene oxide found *M. pulmonis* infection not related to chemical exposure, but affected survival, yet lymphomas/leukemias were not increased
  - Since respiratory infections occur in old rats, and in most RI bioassays, but leukemia/lymphoma are only reported in a few (8/112), the link is unlikely

Does Infection, Not Aspartame, Explain Lymphomas/Leukemias?

- Lymphoma/leukemia in two aspartame studies
- Positive significant trend in males and females, significant increase in females at 5 doses (first study)
- Significant d-r increase in females, especially high dose ($p<0.01$) and in high dose males (second study)
- Controversy is quantitative, not qualitative
- All animals were housed in the same room (personal communication, M. Soffriti)

What About Significant Increases in Tumors Other Than Lymphomas?

Diagnoses of these cancers are not at issue

- Transitional-cell *carcinomas of renal pelvis/ureter* in female rats
- Malignant *schwannomas* in male rats
- *Mammary cancers* in female rats after perinatal through adult exposure
- *Hepatocellular* and *alveolar/bronchioloar carcinomas* in male mice after perinatal-through-adult exposure

Using IARC and EPA criteria, these results – three studies, both genders, and multiple sites – provide <u>unequivocal evidence</u> aspartame is carcinogenic in animals, and possibly or probably carcinogenic in humans

Kidney Tumors

- Transitional-cell carcinomas of renal pelvis/ureter are **highly significant** and **extremely rare** in controls.
  - In 17 studies using 2,669 control S-D rats, they were found in 1 male and 1 female
  - In 10 studies using 1,060 control F-344 rats, they were found in 1 male.
    - Source: Toxicol Pathol 1991;19(3):27-9
- They were found in 21/1500 aspartame treated animals, versus none in controls.
- Carcinomas in females: positive trend ($p<0.05$), and significant increase ($p<0.05$) in high dose females

Kidney Tumors - Continued

- Furthermore, statistically significant increases of dysplastic lesions + carcinomas of renal pelvis/ureter were seen in the four top doses, with a positive trend in females ($p<0.01$).
- “The occurrence of lesions presumed to be preneoplastic may in certain instances aid in assessing the biological plausibility of any neoplastic response observed.” (IARC)
- Chemical-induced rarely occurring kidney tumors are considered clear evidence of carcinogenicity.

What About Signficant Increases in Tumors Other Than Lymphomas?

- “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 ….” (IARC Preamble)

- “The ANS Panel … and EFSA … concluded that the hepatic and pulmonary tumour incidences ….all fall within their own historical control ranges … Based on these data, the Panel concluded that the results … do not provide evidence for a carcinogenic effect of aspartame in mice.”

What About Significant Increases in Tumors Other than Lymphomas?

"The ANS Panel noted that the only **consistent** findings reported by the authors in the two rats studies were an increased incidence of lymphomas/leukemias …." BUT **consistency should not have been expected** and lack of consistency is not a reason to discount the results:

- The first study was nearly 4 times larger and used a wider range (including higher) doses than the second and was thus much more capable of detecting rare tumors (kidney)
- One study included in utero exposure and the other did not. Different exposure scenarios produce different cancer patterns (e.g., Exposure of women to DES increases risk of breast cancer, but in utero exposure increases risk of cervical/vaginal cancer in their daughters)

What About Significant Increases in Tumors Other Than Lymphomas?

- The National Toxicology Program PWG on the first RI rat study of aspartame wrote “cases diagnosed as malignant schwannoma of the cranial nerve were generally confirmed by the PWG,” although some members “preferred a diagnosis of sarcoma, NOS (not otherwise specified)”
  - Both are evidence of carcinogenicity

Do Studies That Did Not Find Cancer Outweigh Studies That Did? (No)

- Searle long-term studies: Suffice it to say they fail to meet current standards and lack statistical power.
- NTP transgenic studies: no longer used for cancer evaluation screening; not reliable
- Lim study: Aspartame wasn't approved until subjects were in their late 30s/40s/50s or older. Exposures early in life are likely to be much more critical. See EPA Guidance (2005). Only five year follow-up. Other major weaknesses

Do Studies That Did Not Find Cancer Outweigh Studies That Did? (No)

- To conclude lack of carcinogenicity, IARC requires multiple, mutually consistent, adequately powered studies covering the full range of human exposures that exclude with reasonable certainty bias, confounding, and chance and provide individual and pooled estimates of risk near unity with narrow confidence intervals.
- “latent periods substantially shorter than 30 years cannot provide evidence for lack of carcinogenicity” (IARC)

Are EFSA's Conclusions Sound and Supported By the Science?

- IARC: "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. … "

Conclusions

- EFSA failed to consider highly relevant scientific evidence
- EFSA's conclusions are not sound and not supported by the science; they contradict established criteria and principles by IARC, NTP
- EFSA overlooks significant flaws in studies finding no adverse effects and dismisses significant findings in studies that find adverse effects without basis

What Should EFSA Do Now?

- Decide on role/purpose/mission
  - Justify past evaluations, protect continued use of aspartame, whitewash concerns OR
  - Outline areas of uncertainty, protect consumers
- Obtain missing information and then assess all relevant scientific evidence
- Do not rely on previous assessments
- Ensure objectivity in every aspect
- Decide on criteria and apply them consistently to all studies and provide thorough analysis

What Conclusions Can We Draw?

Using IARC and EPA criteria, the results from three independent , well-designed and executed animal studies showing tumors in both genders and multiple sites provide unequivocal evidence that aspartame is carcinogenic in animals, and possibly or probably carcinogenic in humans. The finding in a recent epidemiological study of a slight increase in incidence of a tumor type seen in two animal studies lends further support to this conclusion.