SWEETENERS: DO THEY BEAR A CARCINOGENICITY RISK
ILSI Sweeteners Workshop
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The carcinogenic effects of aspartame:
The urgent need for regulatory re-evaluation

Rat Study Shows Cancer, Aspartame Link
WebMD.com - Nov. 18, 2005
http://www.webmd.com/cancer/news/20051118/rat-study-shows-cancer-aspartame-link#1

Aspartame linked to vision loss, cancer and other illnesses
Natural News - November 13, 2016

Splenda "Officially" Linked To Cancer
EpicTimes.com - Mar 14, 2016
http://www.epictimes.com/03/14/2016/splenda-linked-to-cancer/
Aspartame radically increases chances of cancer
AmazingHealth.com – Undated
http://amazinghealth.com/11.06.08-aspartame-radically-increases-chances-of-cancer

Is Splenda linked to cancer risk? Study ties sucralose to leukemia, tumours
GlobalNews.com - March 16, 2016

An Alarming New Study Is Linking Excessive Splenda Usage to Cancer
PopSugar.com - March 11, 2016
AGENDA

• Introduction
  • High Intensity Sweetener Characteristics
    - Structure/sweetness potency/how are they handled
    - Types of products

• How is the Carcinogenicity of a Sweetener Determined?
  • Genetic toxicity studies
  • OECD/Redbook GLP-compliant rodent studies
  • Post-marketing epidemiology studies
• Carcinogenicity of High Intensity Sweeteners
  • Aspartame, Neotame, Advantame
  • Sucralose
  • Saccharin
  • Acesulfame K
  • Cyclamate
  • Steviol glycosides

• Recent Controversies
  • Rodent studies performed by the Ramazzini Institute (Italy)
    • Alleged carcinogenic activity of aspartame and sucralose

• Summary and Conclusions
STRUCTURES, SWEETNESS, PRODUCTS
ASPARTAME AND ACESULFAME K

**Aspartame**
- 200x sweeter than sucrose
- FDA approved

**Acesulfame K (Ace-K)**
- 200x sweeter than sucrose
- FDA Approved
NEOTAME AND ADVANTAME

Neotame
- 7,000 to 13,000 x sweeter than sucrose
- FDA Approved

Advantame
- 20,000x sweeter than sucrose
- FDA Approved
CYCLAMATE AND SACCHARIN

Cyclamate
- 30x sweeter than sucrose
- FDA banned

Saccharin
- 300x sweeter than sucrose
- Marketed in the U.S. as a sweetener for around 100 years
Sucralose
• 600x sweeter than sucrose
• FDA approved

Stevioside (a member of the steviol glycoside family)
• Around 300x sweeter than sucrose
• FDA accepted in 2008

The differences in the structures of the individual sweeteners and the fact that they are handled very differently following oral administration questions how this group of materials could be viewed as being carcinogenic.
<table>
<thead>
<tr>
<th>Sweetener</th>
<th>Regulatory Status</th>
<th>Examples of Brand Names Containing Sweetener</th>
<th>Acceptable Daily Intake (ADI) milligrams per kilogram body weight per day (mg/kg bw/d)</th>
<th>Number of Tabletop Sweetener Packets Equivalent to ADI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acesulfame Potassium (Ace-K)</td>
<td>Approved as a sweetener and flavor enhancer in foods generally (except in meat and poultry)</td>
<td>Sweet One® Sunett®</td>
<td>15</td>
<td>23</td>
</tr>
<tr>
<td>Advantame</td>
<td>Approved as a sweetener and flavor enhancer in foods generally (except in meat and poultry)</td>
<td>Nutrasweet® Equal® Sugar Twin®</td>
<td>50</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td><strong>21 CFR 172.803</strong></td>
<td></td>
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</tr>
<tr>
<td>Aspartame</td>
<td>Approved as a sweetener and flavor enhancer in foods generally</td>
<td>Nutrasweet® Equal® Sugar Twin®</td>
<td>50</td>
<td>75</td>
</tr>
<tr>
<td>Neotame</td>
<td>Approved as a sweetener and flavor enhancer in foods generally (except in meat and poultry)</td>
<td>Neutame®,</td>
<td>0.3</td>
<td>23</td>
</tr>
<tr>
<td>Saccharin</td>
<td>Approved as a sweetener only in certain special dietary foods and as an additive used for certain technological purposes</td>
<td>Sweet and Low® Sweet Twin® Sweet’N Low® Necta Sweet®</td>
<td>15</td>
<td>45</td>
</tr>
<tr>
<td>Certain high purity steviol glycosides purified from the leaves of <em>Stevia rebaudiana</em> (Bertoni) Bertoni</td>
<td>≥95% pure glycosides Subject of GRAS notices for specific conditions of use</td>
<td>Truvia® PureVia® Enliten®</td>
<td>4**</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td><strong>GRAS Notice Inventory</strong> (<a href="http://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices">http://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices</a>)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucralose</td>
<td></td>
<td>Splenda®</td>
<td>5</td>
<td>23</td>
</tr>
</tbody>
</table>
SWEETENED PRODUCTS

Saccharin
Sucralose
Stevia based sweetener
Acesulfame K/Sucralose
Aspartame
Neotame
Acesulfame K
Advantame
HOW IS THE CARCINOGENICITY OF HIS ASSESSED?
CARCINOGENICITY ASSESSMENT IS A DATA DRIVEN PROCESS

- Knowledge of metabolism and structure activity relationships
  - Do structures resemble known carcinogens?
- Results of *in vitro* and *in vivo* genetic toxicity studies
  - Do the sweeteners interact with or damage DNA?
- Results of 2-year studies in rodents at dosages multiples of the estimated human intake
  - Do sweeteners cause increase in any individual tumor types?
- Human data
  - Is there any evidence from epidemiology studies that consumption of HIS increases the risks for any type of cancer?
CARCINOGENICITY ASSESSMENT IS A DATA DRIVEN PROCESS

- There is a hierarchy of data in a “weight-of-the-evidence” type assessment

  1) Prior to marketing, results of rodent studies of 2-years duration conducted according to Good Laboratory Practice (GLP) guidelines and internationally recognized regulatory guidelines for the conduct of these studies (i.e., FDA Redbook, OECD). Considered the “Gold Standard”

  2) Decades after marketing, high quality epidemiology studies, designed such they can detect causal associations (as opposed to correlations) with documented exposure to HIS and incidence of cancer, can be useful

    • Typically need prospective studies (looking forward) as opposed to retrospective studies (observational in nature, looking in the past) that are randomized and double blind

    • Epidemiology studies are of no benefit prior to marketing of a food ingredient/additive
SWEETENER CARCINOGENICITY OUTCOMES
Aspartame, and to a lesser extent cyclamates, saccharin, and sucralose have been studied, either individually, or more commonly, as a group of “high intensity sweeteners” for potential carcinogenic effects in humans.

By and large these studies have reported little, if any, association of HIS as a group or individually with increased cancer risk.

Overall, the epidemiology data are of limited usefulness for the assessment of carcinogenicity of HIS due to:

a. No “gold standard” prospective, double blind studies, with documented or quantified exposure to HIS exist for any HIS

b. Exposures to HIS are so low as to preclude detection of any carcinogenic effect from background

c. It is not possible to isolate or quantify exposures to individual HIS

d. Many studies were subject to various biases (selection and recall) which confound study interpretation
THE WAY FORWARD – CARCINOGENICITY STUDIES IN RODENTS

• Given epidemiology studies lack of utility, and the inability to conduct such studies on new food ingredients/additives; the results of chronic studies in rodents therefore, serve as the main basis to assess carcinogenic potential

• The design and proper conduct and reporting of cancer studies in rodents is described in various regulatory guidelines, including FDA’s Redbook, OECD Test Guideline 451 “Carcinogenicity Studies”, and OECD GLP Guidelines. These guidelines provide specific recommendations in regards to:
  a. study duration (2 years)
  b. number of animals per group (50 to 60/sex/group)
  c. dose selection
  d. parameters to be assessed (hematology, clinical chemistry, etc.)
  e. tissues to be examined histopathologically
  f. types of appropriate statistical analysis
CARCINOGENICITY OF INDIVIDUAL HIS IN RODENTS
The most extensively studied HIS, has been evaluated in 5 guideline studies (3 conducted by Searle and 2 by Ajinomoto - 4 in rats and 1 in mice)
- doses ranged up to 4 to 8 g/kg bw/day in rats and to 4 g/kg bw/day in mice
- one of the rat studies included in utero and lactation phases of exposure
- initial rat study showed non-statistically significant increase in brain tumors in the highest dose group (i.e., 1/40 vs 0/58 in males and 3/39 vs 0/59 in females)
- additional sectioning of the brain in all dose groups including controls showed no overall increase in brain tumor incidence in any dose group
- 3 subsequent guideline rat studies, and an additional study in mice, showed no increase in the incidence of brain tumors
- in all 5 studies there was no indication of any effect of aspartame treatment on the incidence of any other tumor type; 3 subsequent transgenic mice assays conducted by NTP also negative

Conclusion: There is no evidence of carcinogenicity of aspartame in rodent studies
NEOTAME AND ADVANTAME

• Neotame
  - mice, 70/sex/group, dosed at up to 4 g/kg bw/day – non-statistically significant increase in liver adenomas and of lung carcinomas in males and females, respectively, but of not biological significance as no increases when tumor types were appropriately combined (adenoma plus carcinoma) and given there were no pre-neoplastic changes
  - rats, 75/sex/group, in utero, dosed at up to 1 g/kg bw/day – no neoplastic effects

• Advantame
  - mice, 64/sex/group, dosed at up to 50,000 ppm diet – no neoplastic effects
  - rats, 55/sex/group, in utero, dosed at up to 50,000 ppm diet – no neoplastic effects

Conclusion: These aspartame derivatives are not carcinogenic in rodents
ACESULFAME K

- **Mice**, 100/sex/group, dosed at up to 30,000 ppm diet for 80 weeks – no neoplastic changes attributable to treatment

- **Rats**, 60/sex/group, *in utero*, multigenerational, dosed at up to 30,000 ppm – incidence of pulmonary lymphoreticular tumours was relatively high in high-dose groups; only achieved statistical significance in females

- **Rats**, repeat study in a strain (Wistar vs CIVO) with a lower background incidence of pulmonary tumors, 60/sex/group, *in utero*, dosed at up to 30,000 ppm, 120 weeks – no neoplastic effects

NTP conducted further studies in 2 strains of transgenic mice, 30,000 ppm diet, 9 months - no increase in neoplasms

**Weight-of evidence: Acesulfame K is not carcinogenic to rodents**
CYCLAMATE

- Cyclamate originally studied in combination with saccharin, as a sodium or calcium salt, has been evaluated in several older rodent carcinogenicity studies over the last 60 years (not up to today's standards).

- Several older studies exist for cyclamate and saccharin mixtures.

- Based on the numerous studies conducted, even though many were not conducted to today's standards, there is no evidence of carcinogenicity of cyclamate or its metabolic product cyclohexylamine.

- Takayama et al. (2000) conducted a carcinogenicity study over more than 20 years in monkeys fed cyclamate at 100 and 500 mg/kg bw/day, 5 days per week. No evidence of carcinogenic potential.

IARC, on the basis of inadequate evidence in both humans and experimental animals, concluded that cyclamates are not classifiable as to their carcinogenicity to humans (Group 3)

Weight-of evidence: Cyclamate is not carcinogenic to laboratory animals (Bopp et al., 1986)
Like aspartame, saccharin has been extensively studied in rodent toxicology and carcinogenesis studies

- At high doses, generally 3% or more in the diet, saccharin was found to be associated with development of tumors of the urinary bladder in rats

  - Additional mechanistic studies revealed that the tumors developed as a result of rat-specific mode of action.

    a. Rats have a unique combination of high pH, high calcium phosphate, and high protein levels in the urine.
    b. Microcrystals form that damage the lining of the bladder
    c. The bladder responds by overproducing cells leading to tumor formation
    d. No evidence of carcinogenic effect in a long-term monkey study

IARC, concluded that: “sodium saccharin produces urothelial bladder tumours in rats by a non-DNA-reactive mechanism that... is not relevant to humans because of critical interspecies differences in urine composition.”

Conclusion: Saccharin is carcinogenic to the rat, but via a mechanism of no relevance to humans
The carcinogenicity of sucralose has been evaluated in each of rats and mice in 2 year studies, the results of which are published (Mann et al., 2000a,b)

- **mice**, 72/sex/group, dosed at up 30,000 ppm in the diet – no effects on tumor incidence in treated groups relative to controls

- **rats**, 50/sex/group, *in utero*, dosed at up to 30,000 ppm in the diet – “There were no statistically significant increases in the incidence of any neoplasm, and sucralose did not affect the types of tumours observed” (Mann et al., 2000)

**Conclusion**: Sucralose is not carcinogenic to rodents
STEVIO GLYCOSIDES

Steviol glycosides are all metabolized to steviol prior to absorption, hence data for one glycoside can characterize the carcinogenic potential of all steviol glycosides. Carcinogenicity of stevioside has been evaluated in 2 published studies in rats (Toyoda et al., 1997; Xili et al., 1992)

- **Toyoda et al. (1997)**, 50/sex/group, dosed at up 50,000 ppm in the diet – no increases in neoplasms; decreased incidence of mammary adenomas in females

- **Xili et al. (1992)**, 45/sex/group, dosed at up to 12,000 ppm in the diet – no treatment-related increases in any neoplasm type

**Conclusion:** Steviol glycosides are not carcinogenic to rodents
SUMMARY OF RODENT CARCINOGENICITY DATA

Each of the HIS, including aspartame, acesulfame K, neotame, advantame, cyclamates, saccharin, sucralose, and steviol glycosides, have been subject to extensive testing for carcinogenicity in chronic rodent toxicity studies.

Most using protocols as per official guidelines and conducted to GLP

All, except saccharin show no evidence of carcinogenicity in rodents

Saccharin produce bladder tumors in rats at high dietary concentrations, but these tumors form by a rat-specific mechanism and hence do not indicate a carcinogenic risk to humans, a conclusion echoed by IARC
RECENT CONTROVERSIES
Researchers (Soffritti, Belpoggi et al.) at the Ramazzini Institute in Bologna, Italy have reported on 3 rodent carcinogenicity studies with aspartame (2 in rats and 1 in mice) and on 1 for sucralose (in the mouse). All have alleged a carcinogenic effect of these sweeteners using non-standard protocols.

**Aspartame (rats)**

**Belpoggi et al. (2006)** – 100-150/sex/group, treated for life at up to 100,000 ppm in the diet. Reported: increased incidence of malignant tumor-bearing animals, an increase in lymphomas-leukemias, an increase in tumors of the renal pelvis/ureter in females, and an increased incidence of tumors of the peripheral nerves, with a positive trend in males.

**Soffritti et al. (2007)** – 70-95 males & females/group, in utero, exposed up to 2,000 ppm in the diet for life. Reported a significant dose-related increase of malignant tumor-bearing males, a significant increase in incidence of lymphomas/leukemias in high-dose animals, and a significant dose-related increase in incidence of mammary cancer in females.
Aspartame (mice)

Soffritti et al. (2010) – 62-122/sex/group, treated prenatally through to end of life at up to 32,000 ppm in the diet. Reported a significant dose-related increased incidence of hepatocellular carcinomas and of alveolar/bronchiolar carcinomas, both in males.

Sucralose (mice)

Soffritti et al. (2010) – 64-117/sex/group, treated prenatally through to end of life at up to 32,000 ppm in the diet. Reported a significant dose-related increase in total malignant tumors in males and significant dose-related increase in leukemias/lymphomas also in males.
Shortcomings/Flaws of the Ramazzini Studies

- Not designed to GLP-, Redbook/OECD-compliant protocols
- Lifetime dosing has been critiqued (EFSA, US FDA) for producing uninterpretable results due to rapid increase in neoplasm development beyond the age of 2 years
- Apparent lack of randomization of animals (size of studies appears to preclude such randomization) (noted by EFSA)
- Lack of information on the nature of the diets used (not stated in their publications)
- Rats and mice used by Ramazzini have a high and variable spontaneous incidence of leukemia/lymphoma that has been related to infection/inflammation (confounds study interpretation) (EFSA, US EPA/NTP Re: aspartame studies)
- Use of poorly described and/or inadequate statistical tests and incorrect levels of significance (e.g., trend vs pair-wise; significance levels for common vs uncommon tumor [p=0.01 vs 0.05] based on FDA guidance (FDA, 2001)
- Lumping all tumors, malignant, benign, and malignant + benign, rather than by tissue of origin, for analysis is not appropriate (EFSA, NTP Re: aspartame studies)
LIFETIME RODENT STUDIES CONDUCTED BY THE RAMAZZINI INSTITUTE (ITALY)

Shortcomings/Flaws of the Ramazzini Studies (cont’d)

• Dose-response relationships often absent in alleged response

• Effects often reported only in one sex (EFSA Re: aspartame)

• Documented inconsistencies in diagnoses (e.g., leukemia vs infection) (EFSA, EPA Re: aspartame studies)

• Tabulated data does not match alleged “tumorigenic effects” (e.g., sucralose study)

• Limited or no external peer-review of the pathology slides (EFSA, US FDA Re: aspartame studies)

• The Ramazzini Institute has a history of reporting alleged carcinogenic effects of substances, including Coca-Cola (Belpoggi et al., 2006), and methanol (Soffritti et al., 2002), for which there is otherwise no evidence of carcinogenic activity in rodents.
WHAT DO THE RAMAZZINI INSTITUTE STUDIES MEAN?

The results are uninterpretable and unreliable due to flaws in study design, conduct, and reporting as well as a result of the use of rats and mice with a high and variable incidence of leukemia/lymphoma that may be due to, or is difficult to differentiate from, infection and inflammation.

The results of the Ramazzini Institute studies need to be placed into context with the results of core guideline studies conducted to regulatory standards of the time:

- There is no evidence of carcinogenicity of aspartame (5 rodent studies and 3 transgenic mouse assays) or of sucralose (2-year cancer studies in each of rats and mice).
- Conclusions from Regulatory Authorities – do not consider either aspartame or sucralose to be carcinogenic in rodents or have a carcinogenic risk to humans.

The Ramazzini Institute studies provide no credible evidence that either aspartame or sucralose is carcinogenic.
SUMMARY AND CONCLUSIONS
SUMMARY AND CONCLUSIONS

- High intensity sweeteners are approved for use in food products throughout the world, including aspartame, neotame, advantame, cyclamate, saccharin, acesulfame K, sucralose, and steviol glycosides.

- The potential carcinogenicity of food ingredients/additives prior to marketing is mainly achieved through testing in rats and mice using standardized, internationally recognized protocols.

- The rodent carcinogenicity studies on available high intensity sweeteners shows no evidence of carcinogenicity except for saccharin.
  - Saccharin produced bladder tumors at high doses in rats, but these form as a result of a mechanism that is of no relevance to human.
SUMMARY AND CONCLUSIONS

- Recently, the Ramazzini Institute has conducted a series of lifetime carcinogenicity studies on aspartame (2 rat studies 1 mouse study) and sucralose (1 mouse study), alleging carcinogenic effects of these sweeteners.
  - The results are uninterpretable and unreliable due to flaws in study design, conduct, and reporting.
- There is no substantive epidemiological evidence of carcinogenic effects of marketed high intensity sweeteners.
- The carcinogenicity database available for currently approved high intensity sweeteners demonstrates a lack of carcinogenic potential.
  - Supported by results of genetic toxicity data and knowledge of pharmacokinetics, metabolism, and chemical structure.
  - A viewpoint endorsed by JECFA and all other regulatory authorities that have reviewed the data (US FDA, EFSA, FSANZ, and Health Canada).
  - IARC has reviewed cyclamate and saccharin and determined that they are non-carcinogenic.
“Sweeteners” bear no carcinogenic risk even when exposure levels are several orders of magnitude greater than the anticipated daily human ingestion levels.”
THANK YOU!

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