Prenatal and lifespan exposure enhances the sensitivity of carcinogenicity bioassays: the cases of artificial sweeteners aspartame and sucralose

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Early life antecedents of cancer
Early life antecedents of cancer

- Carcinogenicity bioassays in rodents have long been recognized and accepted as valuable and precious predictors of potential cancer hazards in humans.

- Regulatory agencies recommend:
  - 2-year carcinogenicity bioassays (104 wks)
  - starting at 6-8 weeks of age until 110 weeks
    (which represents about 2/3 of the animals’ lifespan)

- A standard 2-year bioassay cannot elucidate the life-span carcinogenic potential of exposures starting from prenatal life.

- For agents to which the majority of the population is exposed, the bioassay should be conducted on large groups of animals exposed from fetal life until natural death in order to increase their sensitivity and their potential to detect and assess carcinogenic risks.
Aspartame
Aspartame (APM): production and use

- 18,000 tons produced as of 2007
- second artificial intense sweetening agent after saccharin
- present in more than 6,000 products (> 500 drugs)
- hundreds of millions of consumers worldwide
- admitted daily intake (ADI) for humans is 40 mg/Kg b.w. in Europe and 50 mg/Kg b.w. in USA
- **Metabolism**: Apm is metabolized in the GIT as aspartic acid, phenylalanine and methanol, both in humans and animals.

- **Genotoxicity**: It has not been proven genotoxic in the *in vitro* and *in vivo* tests performed. Formaldehyde is genotoxic.

- **Carcinogenicity**:
  
  **1970s and 1980s**: Bioassays on Sprague-Dawley rats, Wistar rats and Swiss mice proved negative.

  **2001**: Studies performed by NTP using transgenic mice models were also negative.
### RI integrated project on APM administered with the feed

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Animals</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Species</td>
<td>Age at start</td>
</tr>
<tr>
<td>I</td>
<td>S-D rats</td>
<td>8 weeks</td>
</tr>
<tr>
<td>II</td>
<td>S-D rats</td>
<td>Fetal</td>
</tr>
<tr>
<td>III</td>
<td>S-D rats</td>
<td>Fetal</td>
</tr>
<tr>
<td>IV</td>
<td>S-D rats</td>
<td>Fetal</td>
</tr>
<tr>
<td>V</td>
<td>Swiss mice</td>
<td>Fetal</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Materials and conduct

Experimental conduct:

- APM was administered with feed
- Water and food consumption
- Body weight
- Clinical control
- Complete necropsy
- Histopathology: Full evaluation of all tissues and organs of each animal of each group
- Statistical evaluation: Cochran Armitage; poly-K test; Cox proportional hazard model
## Summary of the carcinogenic effects of APM in rodents

<table>
<thead>
<tr>
<th>Species</th>
<th>Age at start</th>
<th>Lymph/leuk</th>
<th>Kidneys</th>
<th>Nervous sys.</th>
<th>Mammary</th>
<th>Lung</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-D rats</td>
<td>8 weeks</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+ DR</td>
<td>+ DR</td>
<td>+ DR</td>
</tr>
<tr>
<td>S-D rats</td>
<td>fetal</td>
<td>+</td>
<td>+</td>
<td>+ DR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-mice</td>
<td>fetal</td>
<td></td>
<td></td>
<td></td>
<td>+ DR</td>
<td>+ DR</td>
<td></td>
</tr>
</tbody>
</table>

+= significantly increased; DR= Dose-related; CP= Carcinomas of the pelvis & ureter; MS= Malignant Schwannomas; ADC= Adenocarcinomas; HCC= Hepatocellular carcinomas;
Comparison of the cumulative prevalence of HLRN neoplasia by age of death in female rats

Prenatal exposure

Postnatal exposure
The RI results motivated epidemiologists from Harvard to investigate on the association between APM and cancer (lymphoma and leukemia)

Analysis of the data bank from approximately 77,000 women (nurses) and 48,000 men (doctors, pharmacists, etc.) led the authors to conclude:

- In men, the risk of NHL significantly increased for subjects who consumed ≥ 1 serving diet soda/day. The risk was greater for consumption of ≥ 2 servings diet soda/day and increased linearly with increased consumption of diet soda

- In men the risk of multiple myeloma increased linearly with increased consumption and was significantly elevated for subjects who consumed ≥ 1 serving diet soda/day
Sucralose
Sucralose: use and general information

- It was accidentally discovered in 1976
- It is produced from sucrose by replacing 3 OH groups with Cl
- It is 600 times as sweet as sucrose and it is present in more than 4,500 products, including many drugs
- Its production in 2018 is expected to be 15,000 tons
- It is minimally absorbed after oral ingestion
- It has been shown to be non genotoxic
- Carcinogenicity bioassays in rats and mice performed by the manufacturer did not show any evidence of carcinogenic effects
- ADI for humans is 5 mg/Kg b.w. in US and 15 mg/Kg b.w. in Europe
Sucralose experiment on mice: the plan

<table>
<thead>
<tr>
<th>Age at start</th>
<th>Animals</th>
<th>Dose/group, ppm (mg/Kg b.w.)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Fetal</td>
<td>No. males</td>
<td>117</td>
</tr>
<tr>
<td>Fetal</td>
<td>No. females</td>
<td>102</td>
</tr>
<tr>
<td>Total:</td>
<td></td>
<td>843</td>
</tr>
</tbody>
</table>

<sup>a</sup>The intake in mg/Kg b.w. was calculated considering the average weight of a mouse for the duration of the experiment as 40 gr and the consumption of feed equal to 5 gr/day for both sexes.
Sucralose experiment on mice: Survival Function for Kaplan-Meier Estimation

Male

Females

Dose=0,  Dose=500,  Dose=2000,  Dose=8000,  Dose=16000,
Swiss mice experiment on Sucralose: first results

<table>
<thead>
<tr>
<th>Animals</th>
<th>Incidence of malignant tumor-bearing animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ppm in feed (mg/Kg b.w.)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>56.4*#</td>
</tr>
<tr>
<td>Females</td>
<td>67.6</td>
</tr>
</tbody>
</table>

* significant (p≤0.05) Cox Proportional Hazard Model;
# near the control incidence is the p-value (p≤0.05) associated with Cox Regression Model for the analysis of the trend
### Incidence of leukemias in males

<table>
<thead>
<tr>
<th>ppm in feed (mg/Kg b.w.)</th>
<th>0</th>
<th>500</th>
<th>2,000</th>
<th>8,000</th>
<th>16,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>(62.5)</td>
<td>(250)</td>
<td>(1,000)</td>
<td>(2,000)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.7##</td>
<td>5.3</td>
<td>17.5**</td>
<td>10.6*</td>
<td>15.7**</td>
<td></td>
</tr>
</tbody>
</table>

* significant (p≤0.05) using Cox Proportional Hazard Model;
** significant (p≤0.01) using Cox Proportional Hazard Model;
## near the control incidence is the p-value (p≤0.01) associated with Cox Regression Model for the analysis of the trend
The cases presented show that long-term bioassays on large groups of animals exposed from fetal life until natural death represent a reliable model to increase the sensitivity and the potential for detecting and assessing carcinogenic risk to humans.

Exposures in prenatal and early life can improve our knowledge of the biological basis of cancer processes and the potential role of such exposures in disease etiology.

Truncating the studies after 2 years, when more than 50% of the animals are still alive, does not allow sufficient latency periods for late tumors to develop and be detected. This is particularly dangerous since in animals, as in humans, most tumors develop in the last part of life.
Concluding remarks

The RI bioassay distinctive features make our studies not only a tool to screen substances on the basis of their carcinogenic potential, but a comprehensive approach to full risk assessment, extremely important for agents to which most of the population are exposed.

The current standard long-term bioassays demanded by Regulatory Agencies may lose important information for extrapolation of data from animals to humans, especially in cases of agents with a long latency time (weak carcinogens).
Regarding APM and sucralose studies

- On the basis of our experimental results and of the Harvard epi-study, we believe that action must be taken to review the present regulations governing the use of food additive.

- The evidence of the carcinogenic effects of sucralose in mice calls urgently for a study on rats, which we are planning to start shortly.

- These are paradigmatic examples for which application of the precautionary principle is surely crucial:

  “We do not have the 100% certainty of a cancer risk, but we have enough information to assume that it may exist”
Thank-you for your attention and…

- Thanks to all the research staff of the Cesare Maltoni Cancer Research Center of the Ramazzini Institute

- Thanks to Dr. D.G, Hoel for his precious collaboration with statistical analysis of the data

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