Ramazzini Institute

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Aspartame: the experimental evidence of cancer risks

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Artificial sweeteners (AS): general information (Part I)

- World Wars I and II mark the introduction of the artificial sweetener saccharin as a substitute for sucrose, its low cost enabling it to prevail
- From the early ’70s the obesity problem grows in industrialized countries due in part to fast food and soft drink consumption
- Concurrently the demand for sweeteners in reduced-calorie foodstuffs increased
- Given the lucrative market for these so-called “dietetic” or “light” products, additional sweeteners emerged, including: aspartame, cyclammate, acesulfame-K, sucralose and neotame

Artificial sweeteners (AS): general information (Part II)

- As the artificial sweetener market expanded in the ’80s and ’90s, concern arose among consumers regarding their safety and possible long-term effects, especially the carcinogenic risk
- Most long-term carcinogenicity bioassays on artificial sweeteners performed on rodents in the past had not been adequately designed to assess the carcinogenic risks (as reported several times by Dr. David Rall, the founder of NTP in the US)

Artificial sweeteners (AS): general information (Part III)

- Because of globalization and the ever-increasing use of artificial sweeteners among billions of people in both industrialized and developing countries, in the late 1990s the Ramazzini Institute began an integrated project of mega-experiments to test the carcinogenic potential of aspartame and sucralose

Aspartame

Aspartame (APM): production and use

- 18,000 tons produced as of 2007
- second artificial intense sweetening agent after saccharin
- 62% of the intense sweetening agents market
- present in more than 6,000 products
- hundreds of millions of consumers worldwide
- average daily intake in US and Europe
  - Projected maximum consumption: 22-34 mg/Kg b.w.
  - Children/women of childbearing age: 2.5-5 mg/Kg b.w.

APM: general information

- ➔ Metabolism: in the GI tract as aspartic acid, phenylalanine and methanol, both in humans and animals
- ➔ Genotoxicity: APM has been shown to be non genotoxic in various tests in vitro and in vivo
- ➔ Carcinogenicity
  - **1970s and 1980s**: studies on Sprague-Dawley rats, Wistar rats and Swiss mice, which we consider inadequate on the basis of the current standards for experimental design and conduct in rodent bioassays
  - **2001**: studies performed by NTP using transgenic mice models

The integrated experimental project of the Ramazzini Institute on Aspartame started in 1997

RI integrated project on APM administered with the feed

	Animals		
Experiment	Species	No.	Status
first	S-D rats	1800	published (2005)
second	S-D rats	470	published (2007)
third	S-D rats	429	ongoing (biophase ended)
fourth	S-D rats	430	ongoing (biophase ended)
fifth	Swiss mice	852	published (2010)
Total		**3981**	

APM: materials and conduct

Experiment phases:

- ➔ APM technical product, > 98.7% purity
- ➔ APM was administered with feed
- ➔ Water and food consumption
- ➔ Body weight
- ➔ Clinical control
- ➔ Complete necropsy
- ➔ Histopathology
- ➔ Statistical evaluation
  - Cochran – Armitage; poly-K test; Cox proportional hazard model

First Aspartame experiment

(Sprague-Dawley rats)

First APM experiment on rats: the plan

Age at start	Animals	dose/group ppm (mg/kg b.w.) [a,b]							
		100,000 (5,000)	50,000 (2,500)	10,000 (500)	2,000 (100)	400 (20)	80 (4)	0 (control)	**TOTAL**
6 weeks	n. males	100	100	100	150	150	150	150	900
8	n. females	100	100	100	150	150	150	150	900
Total		200	200	200	300	300	300	300	1800

[a] Considering the average weight of a rat as 400g, and average food consumption as 20g per day
[b] The treatment lasts for the entire life span

First APM experiment on rats: the results

Significant increased incidence of:

- lymphomas and leukemias in females (dose-related)
- preneoplastic atypical lesions and carcinomas of the renal pelvis and ureter in females (dose-related)
- malignant schwannomas of peripheral cranial nerves in males (dose-related)

Second Aspartame experiment

(Sprague-Dawley rats)

Second APM experiment on rats: the plan

Age at start	Animals	Dose/group,ppm (mg/kg b.w.) [a,b]			
		2,000 (100)	400 (20)	0 (control)	**TOTAL**
Fetal life	n. males	70	70	95	**235**
Fetal life	n. females	70	70	95	**235**
Total		140	140	190	**470**

[a] Considering the average weight of a rat as 400g, and average food consumption as 20g per day
[b] The treatment lasted for the entire life span

Second APM experiment on rats: mammary cancers (%)

Animals	dose/group, ppm (mg/kg b.w.) [a]		
	2,000 (100)	400 (20)	0 (control)
Males (%)	**2.9**	**-**	**-**
Females (%)	**15.7***	**7.1**	**5.3***

[a] p-values associated with the trend test are near the control incidence

* significant ($p<0.05$) using Cox Regression Model

Second APM experiment on rats: lymphomas and leukemias (%)

Animals	dose/group ppm (mg/kg b.w.) [a]		
	2,000 (100)	400 (20)	0 (control)
Males (%)	**17.1***	**15.7**	**9.5**
Females (%)	**31.4****	**17.1**	**12.6****

[a] p-values associated with the trend test are near the control incidence

* significant ($p<0.05$) using Cox Regression Model

** significant ($p<0.01$) using Cox Regression Model

Comparison of lymphomas/leukemias in females: prenatal vs. postnatal exposure

Dose, ppm (mg/kg b.w.)	Females with lymphomas/leukemias (%) [a, b, c]	
	Prenatal exposure	Postnatal exposure
2,000 (100)	**31.4°°**	**18.7#**
400 (20)	**17.1**	**20.0##**
0 (control)	12.6°°	8.7(**#)c

[a] p-values corresponding to pairwise comparison between the controls and the dosed group are near the dosed group incidence.

[B] p-values associated with the trend test are near the control incidence

[c] The p-values associated with the trend test refer to the 7 groups of the first APM experiment

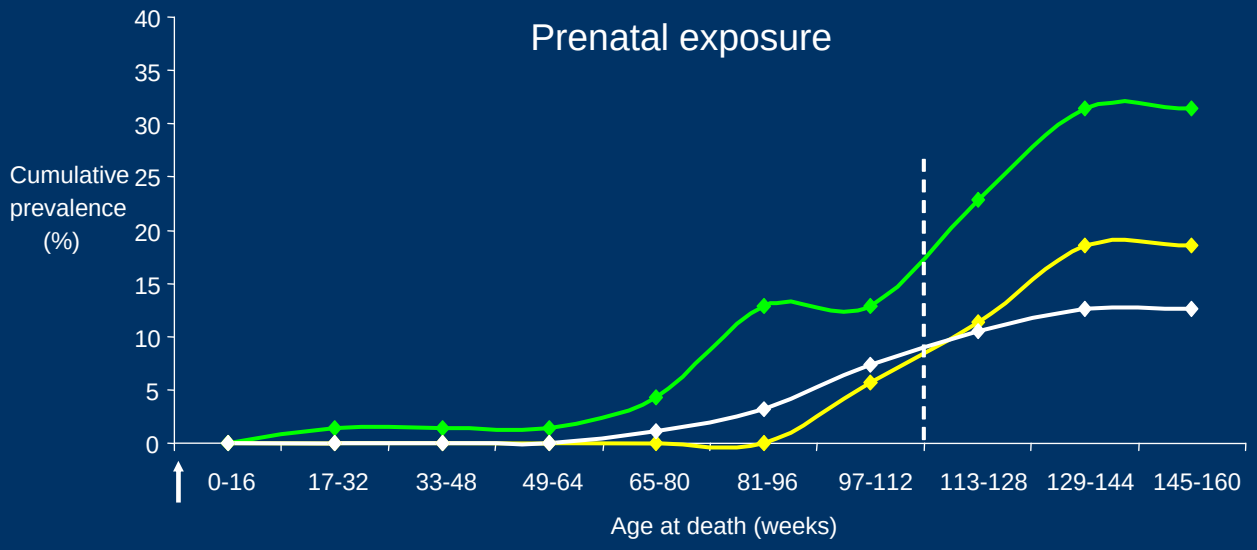
** Statistically significant ($p<0.01$) using the Cochran-Armitage test

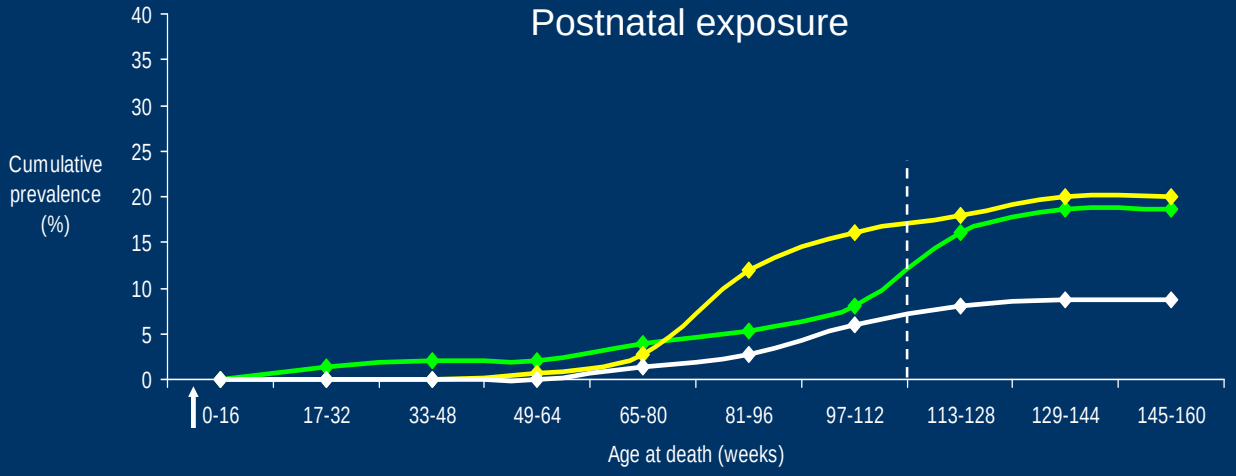
Statistically significant ($p<0.05$) using the poly-k test ($k = 3$)

Statistically significant ($p<0.01$) using the poly-k test ($k = 3$)

°° Statistically significant ($p<0.01$) using the Cox Regression Model

Comparison of the cumulative prevalence of hemolymphoreticular neoplasia in rats by age at death





Start of treatment

2000 ppm

400 ppm

0 ppm (control)

III APM Experiment on Swiss mice

Swiss mice experiment on APM: the plan

Age at start	Animals	Dose/group, ppm [a] (mg/Kg b.w.)					
		32,000 (3910)	16,000 (1920)	8,000 (990)	2,000 (240)	0 (Control)	**TOTAL**
fetal	n. males	83	64	73	122	102	444
fetal	n. females	62	64	62	103	117	408
Total		145	128	135	225	219	852

[a] **The treatment lasts for the entire life span**

Swiss mice experiment on APM: Alveolar Bronchiolar Adenomas (A/BA) and Carcinomas (A/BC) in males[a] (%)

Animals	Tumors	Dose/group, ppm (mg/Kg b.w.)				
		32,000 (3910)	16,000 (1920)	8,000 (990)	2,000 (240)	0
Males	A/BA	7.2	10.9	11.3	8.7	6.8
Males	**A/BC**	**13.3***	**12.5**	**11.3**	**5.8**	**6.0***
Total		**20.5#**	23.4	22.6	14.6	**12.8#**

[a] p-values associated with the trend test are near the control incidence

* significant ($p<0.05$) using the Cox Regression Model

significant ($p<0.05$) using Logistic analysis

Swiss mice experiment: Hepatocellular Adenomas (HA)/ Carcinomas (HCC) in males[a], %

Animals	Tumors	Dose/group, ppm (mg/Kg b.w.)				
		32,000 (3910)	16,000 (1920)	8,000 (990)	2,000 (240)	0
Males	HA	2.4	9.4	6.5	9.7	7.7
Males	**HCC**	**18.1****	**15.6***	**14.5**	**11.7**	**5.1****
	Total	20.5	**25.0**[#]	21.0	21.4	12.8

[a] p-values associated with the trend test are near the control incidence

* significant ($p<0.05$) using the Cox Regression Model

** significant ($p<0.01$) using the Cox Regression Model

[#] significant ($p<0.05$) using Logistic analysis

Summary of the carcinogenic effects of APM in rodents

Significantly increased malignant tumors

Species	Age at start	Lymph/ leuk		Kidneys CP		Nervous sys. MS		Mammary ADC		Lung ADC		Liver HCC	
		M	F	M	F	M	F	M	F	M	F	M	F
S-D rats	8 weeks		+ DR		+ DR		+ DR						
S-D rats	fetal	+	+ DR						+ DR				
S-mice	fetal										+ DR		+ DR

+= significantly increased;
DR= Dose-related;
CP= Carcinomas of the pelvis & ureter;

MS= Malignant Schwannomas;
ADC= Adenocarcinomas;
HCC= Hepatocellular carcinomas;

Conclusions on the APM project

In our experimental conditions APM has been shown to induce a significantly increased incidence of malignant tumors in:

- multiple tissues in male and female rats
- multiple tissues in male mice
- earlier occurrence in treated animals
- **higher incidence and anticipated onset of cancers when the treatment starts from fetal life**

The carcinogenic effects of APM were also shown at dose levels to which humans could be exposed

Conclusive remarks

- On the basis of the results of our experiments, we believe that action must be taken to review the present regulation governing the use of aspartame and sucralose
- This review is particularly necessary if we consider that children and women in childbearing age are the major consumers
- It must be noted that on the basis of our results on APM, the European Food Safety Authority was requested by the European Parliament to review all the scientific literature available on Aspartame in order to update the regulation in Europe before the end of this year instead of 2020 as it was planned

Concluding remarks

> This review has been organized with better transparency in order to allow all social parties to express their judgment based on the knowledge of all the data available

> This is the only way to prevent a conflict of interest among the agency consultants which represent the **shadow cone** in relations between science and civil society