

European Foundation of Oncology and Environmental Sciences "B. Ramazzini"

Cesare Maltoni Cancer Research Center



Early exposure to
chemical additives on
food and cancer:
the case of aspartame

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VIENNA

10 June 2007

4° International Conference on Children's Health and the Environment

Beverages and diet products studied at the CMCRC

Products	No. of bioassays	Animals		Status of studies ^a
		Species	No.	
Water in polyvinylchloride bottles	2	Rat ^b	2200	P ^c
Coca-cola	4	Rat ^b	1999	RP
Pepsi-Cola	1	Rat	400	E
Ethyl alcohol (10% v/v)	4	Rat ^b , mouse	1458	P ^d
Sucrose	1	Rat	400	E
Aspartame	6	Rat ^b , mouse	4460	BO, PP ^e
Sucralose	1	Mouse ^b	760	BO
Caffeine	1	Rat	800	E
Vitamin A	5	Rat	5100	PP ^f
Vitamin C	5	Rat	3680	E
Vitamin E	5	Rat	3680	E
Feed sterilized by gamma rads	1	Rat ^b	2000	E
TOTAL	36		26937	

^a P = published; PP = partially published; RP = ready for publication; E = in elaboration; BO = biophase ongoing

^b Treatment started from embryonal life; ^c Maltoni et al. 1997; ^d Soffritti et al. 2002a; ^e Soffritti et al. 2005;

^f Soffritti et al. 1992

Aspartame (APM): production and use

- 16,000 tons produced as of 2004
- 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

APM: intake

average daily intake among **US consumers** (1984-1992):

- general population = 2-3 mg/kg b.w.
- children/women of childbearing age = 2.5-5 mg/kg b.w.

intake similar elsewhere, including **7 EU countries**:

- Denmark (1999) = 4 mg/kg b.w.

APM: regulatory approval

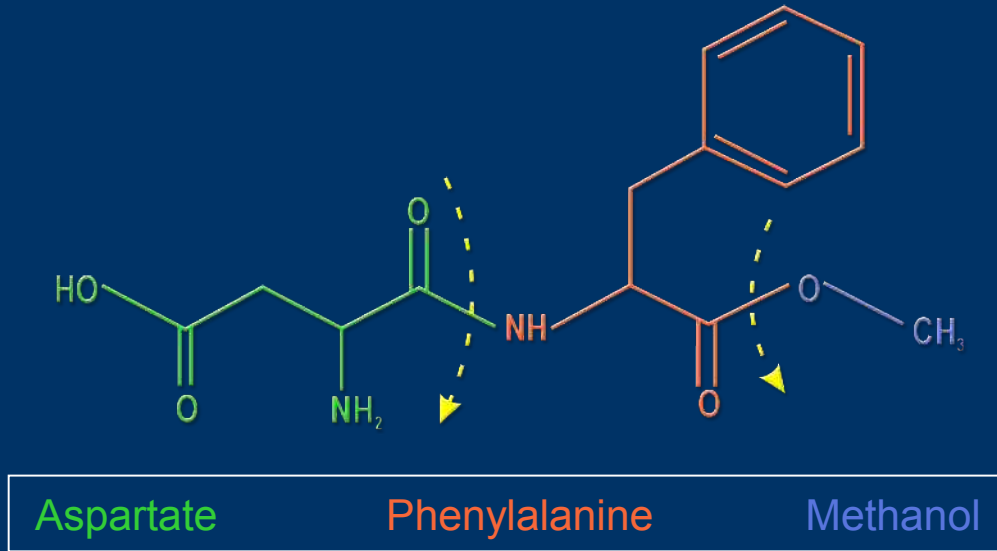
US FDA

- 1974 trade
- 1981 solid foods
- 1983 beverages
- 1996 all foods
- **Acceptable Daily Intake (ADI) = 50mg/Kg bw**

European Union

- 1994 determined safe for all use
- **ADI = 40mg/Kg bw**

APM: metabolism and genotoxicity



- metabolized in the GI tract as **aspartic acid**, **phenylalanine** and **methanol**, both in humans and animals
- the metabolites, once absorbed, enter the blood circulation
- APM has been shown to be **non-genotoxic** in various tests

APM: carcinogenicity

1973. Groups of 40 M and 40 F post-weaning Sprague-Dawley rats treated with APM in feed at doses of 0, 1, 2, 4, 6, 8 g/Kg b.w./day for 104 weeks. 60 M and 60 F served as controls.

No dose-related increase of brain tumors in treated groups.

1974. Groups of 40 M and 40 F Sprague-Dawley rats treated from fetal life and, after weaning, for 104 weeks with APM in feed at doses of 0, 2, 4 g/Kg b.w./day. 60 M and 60 F served as control.

Decrease of brain tumors incidence in treated groups.

APM: carcinogenicity

1981. Groups of **86 M** and **86 F**, 6 week-old Wistar rats treated with APM in feed at doses of 0, 1, 2, 4 g/Kg b.w./day from 6 to 104 weeks of age.

No increase of brain tumors observed.

1981. Groups of **36 M** and **36 F** CD-1 mice treated with APM in feed at doses of 1, 2, 4 g/Kg b.w./day from 6 to 110 weeks of age. 72 males and 72 females served as controls.

No carcinogenic effects observed.

First Experiment on Aspartame



Plan of the first ERF mega-experiment

Animals	Age at start	Number per dose group ppm (mg/kg b.w.) ^{a,b,c}							TOTAL
		100,000 (5,000)	50,000 (2,500)	10,000 (500)	2,000 (100)	400 (20)	80 (4)	0 (control)	
Males	8	100	100	100	150	150	150	150	900
Females	8	100	100	100	150	150	150	150	900

^a Considering the average weight of a rat as 400 g, and average food consumption as 20 g per day

^b Between brackets: the human acceptable daily intake (ADI) equivalent, considering an ADI of 50 mg/Kg b.w. for humans

^c **The treatment lasts for the entire life span**

Materials and methods

Test compound

- purity > 98%
- diketopiperazine < 1.5%
- other impurities < 1.0%

Experiment Conduct

- food consumption
- body weight
- clinical control
- complete necropsy



Incidence of malignant tumor-bearing animals

Animals	ppm in feed (mg/kg b.w.) ^a						
	100,000 (5,000)	50,000 (2,500)	10,000 (500)	2,000 (100)	400 (20)	80 (4)	0 (control)
Males (%)	43.0	38.0	34.0	40.0	32.0	29.3	35.3*
Females (%)	51.0	58.0##	40.0	44.7	46.7	42.7	36.7**

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

* Statistically significant (p<0.05) using Cochran-Armitage test.

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

Incidence of males bearing malignant schwannomas of peripheral nerves

Animals	ppm in feed (mg/kg b.w.) ^b						
	100,000 (5,000)	50,000 (2,500)	10,000 (500)	2,000 (100)	400 (20)	80 (4)	0 (control)
Males (%)	4.0	3.0	2.0	1.3	2.0	0.7	0.7^{*#}

^a **Historical control incidence of malignant schwannomas in males (2,265): 0.4% (range: 0-2.0%)**

^b p-values associated with the trend test are near the control incidence

* Statistically significant ($p < 0.05$) using Cochran-Armitage test.

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

Incidence of preneoplastic lesions with atypia (PLA) and carcinomas (CA) of the renal pelvis and ureter in females

Animals/lesions	ppm in feed (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)
Females/PLA (%)	11.0	7.1	7.0	4.7	4.0	3.3	1.3**
Females/CA (%) ^c	4.0#	3.0	3.0	2.0	2.0	0.7	-
Total (%)	15.0##	10.1##	10.0##	6.7#	6.0#	4.0	1.3 ^{***##}

^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 **Historical control incidence of renal pelvis CA in females (2,274): 0.04% (range: 0-1.0%)**

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

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

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

Incidence of females bearing lymphomas and leukemias^a

Animals	ppm in feed (mg/kg b.w.) ^{b, c}						
	100,000 (5,000)	50,000 (2,500)	10,000 (500)	2,000 (100)	400 (20)	80 (4)	0 (control)
Females (%)	25.0##	25.0##	19.0#	18.7#	20.0##	14.7	8.7***

^a **Historical control incidence of lymphomas and leukemias in females (2,274): 13.3% (range: 4.0-25.0%)**

^b p-values corresponding to pairwise comparison between the controls and the dosed group are near the dosed group incidence.

^c p-values associated with the trend test are near the control incidence

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

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

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Conclusions from the first ERF mega-experiment

APM has a multipotential carcinogenic effect based on:

1. the significant increase of males and females bearing **malignant tumors**
2. the significant increase of **haemolymphoreticular neoplasias** in females
3. the significant increase of the combined incidence of **transitional cell carcinomas** and their precursors (dysplasias) in renal pelvis and ureter in females
4. the significant increase of **malignant schwannomas** peripheral cranial nerves in males

Selected reactions to the first ERF mega-experiment

- Fall '05. US NTP publishes experimental studies on genetically altered mice
- Fall '05. International agencies and governments request complete raw data set from ERF
- Spring '06. US NCI publishes an abstract relative to an epidemiological study
- Spring '06. EFSA issues opinion on ERF mega-experiment
- Winter '06. Aspartame industry comments in EHP

Experimental studies

- October 2005. The 6-month transgenic mice studies conducted by the US NTP do not demonstrate the carcinogenic effects of APM.
- The NTP committee notes in the report that “there is uncertainty whether the study possessed sufficient sensitivity to detect a carcinogenic effect.”

Epidemiological study

- April 2006. US NCI publishes an abstract based on a vast number of self-administered diet questionnaires mailed from 1995 to 1996 to persons aged 50-71. One question concerns the consumption of beverages potentially containing APM during the previous year. The study concludes that APM does not increase hematopoietic or brain cancer risks. The full paper is published in September.
- Given the timeframe of the surveys and the commercialization of aspartame in the 1980s, the subjects' potential use of the sweetener could not have exceeded 10-15 years. It is difficult to think that this limited adult exposure to APM could evidence or exclude a potential carcinogenic risk.

Second Experiment on Aspartame



Plan of the second ERF experiment

Animals	Age at start	Number per dose group ppm (mg/kg b.w.) ^{a,b,c}			TOTAL
		2,000 (100)	400 (20)	0 (control)	
Males	Fetal life	70	70	95	235
Females	Fetal life	70	70	95	235

^a Considering the average weight of a rat as 400 g, and average food consumption as 20 g per day

^b Between brackets: the human acceptable daily intake (ADI) equivalent, considering an ADI of 50 mg/Kg b.w. for humans

^c **The treatment lasts for the entire life span**

Incidence of animals bearing malignant tumors

Animals	ppm in feed (mg/kg b.w.) ^a		
	2,000 (100)	400 (20)	0 (control)
Males (%)	40.0 **	25.7	24.2**
Females (%)	52.9	44.3	44.2

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

** Statistically significant (p<0.01) using Cox Regression Model.

Incidence of animals bearing mammary cancers

Animals	ppm in feed (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

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

Comparison of mammary cancers in females: prenatal vs. postnatal exposure

Dose, ppm (mg/kg b.w.)	Females with mammary cancers (%) ^{a, b}	
	Prenatal exposure	Postnatal exposure
2,000 (100)	15.7*	8.0
400 (20)	7.1	10.7
0 (control)	5.3*	5.3

^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 Statistically significant ($p < 0.05$) using Cox Regression Model

Incidence of animals bearing lymphomas/leukemias

Animals	ppm in feed (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

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

** Statistically 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^{oo}	18.7[#]
400 (20)	17.1	20.0^{##}
0 (control)	12.6 ^{oo}	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 is referred to the 7 groups of first APM experiment

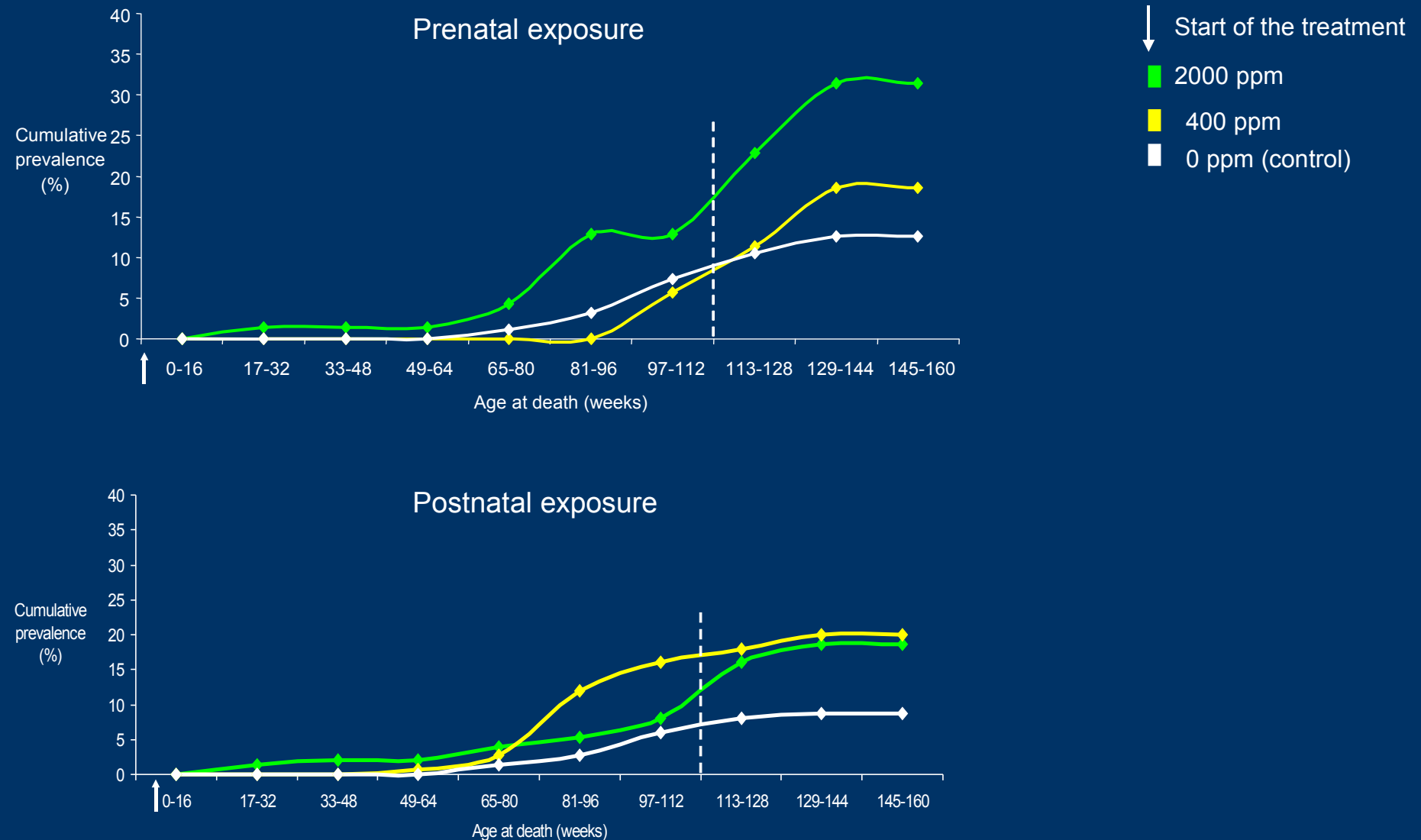
^{**} Statistically significant (p<0.01) using Cochran-Armitage test

[#] Statistically significant (p<0.05) using poly-k test (k = 3)

^{##} Statistically significant (p<0.01) using poly-k test (k = 3)

^{oo} Statistically significant (p<0.01) using Cox Regression Model

Comparison of the cumulative prevalence of hemolymphoreticular neoplasia by age of death



Conclusions from the second ERF experiment

1. The results of the transplacental carcinogenicity bioassay on APM **not only confirm, but also reinforce** our first experimental demonstration of APM's multipotent carcinogenicity
2. The study demonstrates that when lifespan exposure to APM begins during **fetal life**, its **carcinogenic effects are increased**
3. On the basis of the present findings, we believe that a **review of the current regulations** governing the use of APM cannot be delayed in accordance with the guidelines of the **Precautionary Principle**.

Conclusions from the second ERF experiment

4. In addition to considering new scientific data on artificial sweeteners, we must also urgently reevaluate the **adequacy of previous studies**, conducted mainly by industry.

-not only APM, but also saccharin, sucralose, acesulfame K and cyclamates

5. The **potential sincarcinogenetic effects** of blends of artificial sweeteners must also be considered

Comments

- Exposure to carcinogenic agents beginning during developmental life may have **strong carcinogenic effects in adults**.
- Long-term carcinogenicity bioassays are a **good tool** to predict the effects of exposure to carcinogenic agents beginning during developmental life and to make decisions to protect public health.

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