

Aspartame induces lymphomas and leukaemias in rats^a

L'aspartame induce linfomi e leucemie nei ratti

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Summary

Aspartame, a widely used artificial sweetener, was administered with feed to male and female Sprague-Dawley rats (100-150/sex/group), 8 weeks-old at the start of the experiment, at concentrations of 100,000; 50,000; 10,000; 2,000; 400; 80 and 0 ppm. Treatment lasted until spontaneous death of the animals. In this report we present the first results showing that aspartame, in our experimental conditions, causes a statistically significant, dose-related increase in lymphomas and leukaemias in females. No statistically significant increase in malignant brain tumours was observed among animals from the treated groups as compared to controls. Eur. J. Oncol., 10 (2), 107-116, 2005

Key words: aspartame, artificial sweetener, carcinogenesis, rats, lymphoma, leukaemia

Introduction

Aspartame (APM) is a widely used artificial sweetener consumed by hundreds of millions of people around the world^{1,2}. It is found in more than 6,000 products, including soft drinks, chewing gum, candy, yoghurt, table-

Riassunto

L'aspartame, un dolcificante artificiale largamente diffuso, è stato somministrato con il mangime a ratti Sprague-Dawley, maschi e femmine (100-150/sex/gruppo), di 8 settimane di età all'inizio dell'esperimento, a concentrazioni di 100.000; 50.000; 10.000; 2.000; 400; 80 e 0 ppm. Il trattamento è durato fino alla morte spontanea degli animali. In questo articolo vengono presentati i primi risultati che dimostrano come l'aspartame, nelle nostre condizioni sperimentali, causa un incremento statisticamente significativo, dose-correlato, di linfomi e leucemie nelle femmine. Nei gruppi trattati rispetto al controllo non è stato osservato nessun aumento statisticamente significativo dei tumori maligni del cervello. Eur. J. Oncol., 10 (2), 107-116, 2005

Parole chiave: aspartame, dolcificante artificiale, cancerogenesi, ratti, linfoma, leucemia

top sweeteners and some pharmaceuticals such as vitamins and sugar-free cough drops².

Dietary surveys, performed among APM consumers, have shown that the average APM daily intake in the general population ranged from 2 to 3 mg/kg b.w. and was even more in children and pregnant women¹. The Accept-

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able Daily Intake (ADI) both in the US and in Europe is 50 and 40 mg/kg b.w., respectively¹.

In rodents and humans, APM is metabolised in the gastrointestinal tract into three constituents: aspartic acid, phenylalanine and methanol³.

Three long-term feeding carcinogenicity bioassays on APM were performed on rats, and one on mice, during the 1970s. Overall, the carcinogenicity studies were considered negative⁴, but it must be noted that these studies did not comply with the basic requirements which must nowadays be met when testing the carcinogenicity potential of a chemical or physical agent. Because of these limitations, we decided to perform a mega-experiment following the currently accepted Good Laboratory Practices.

In the present paper we are reporting our first results on the incidence of haemolymphoreticular malignancies (lymphomas and leukaemias) and malignant brain tumours.

Materials and methods

The APM used was produced by Nutrasweet and supplied by Giusto Faravelli S.p.A., Milan, Italy. As an active ingredient, its purity was more than 98%. To simulate an assumed daily intake by humans of 5,000; 2,500; 500; 100; 20; 4; or 0 mg/kg b.w., APM was added to the standard Corticella diet, used for 30 years at the laboratory of the Cancer Research Centre (CRC) of the European Ramazzini Foundation (ERF), at concentrations of 100,000; 50,000; 10,000; 2,000; 400; 80; or 0 ppm. APM-treated feed was administered *ad libitum* to Sprague-Dawley rats (100-150/sex/group), 8 weeks old at the start of the experiment, and the treatment lasted until spontaneous death. Control animals received the same feed without APM. The plan of the experiment is shown in Table 1.

Male (M) and female (F) rats from the colony of the CRC were used. This colony of rats has been employed

Table 1 - Long-term carcinogenicity bioassay on aspartame administered with feed supplied *ad libitum* to male (M) and female (F) Sprague-Dawley rats from 8 weeks of age until spontaneous death. Plan of the experiment

Groups No.	Age at start (weeks)	Animals		Treatment			Duration
		Sex	No.	Dose			
				ppm	mg/kg b.w. ^a	Human ADI equivalent ^b	
I	8	M	100	100,000	5,000	100X	Life span
		F	100				
		M+F	200				
II	8	M	100	50,000	2,500	50X	Life span
		F	100				
		M+F	200				
III	8	M	100	10,000	500	10X	Life span
		F	100				
		M+F	200				
IV	8	M	150	2,000	100	2X	Life span
		F	150				
		M+F	300				
V	8	M	150	400	20	0.4X	Life span
		F	150				
		M+F	300				
VI	8	M	150	80	4	0.08X	Life span
		F	150				
		M+F	300				
VII	8	M	150	0	-	-	Life span
		F	150				
		M+F	300				

^a The daily assumption in mg/kg b.w. was calculated considering the average weight of a rat for the duration of the experiment as 400 g, and the average consumption of feed as 20 g per day, both for males and females

^b Considering the Acceptable Daily Intake (ADI) of 50 mg/kg b.w. for humans

for various experiments in the CRC laboratory for nearly 30 years. Data are available on the tumour incidence among untreated Sprague-Dawley rats. These animals were monitored for feed, water consumption, and body weight, for their life span and, at death, underwent complete necropsy and histopathological evaluation (historical controls).

The experiment was conducted according to the Italian law regulating use of animals for scientific purposes⁵.

After weaning, at 4-5 weeks of age, the experimental animals were identified by ear punch, randomised in order to have no more than one male and one female from each litter in the same group, and housed in groups of 5 in

makrolon cages (41x25x15 cm), with stainless-steel wire tops and a shallow layer of white wood shavings as bedding. All the animals were kept in rooms assigned only to this experiment, at $23 \pm 2^\circ\text{C}$ and 50-60% relative humidity.

Mean daily drinking water and feed consumption were measured per cage, and body weight individually, once a week for the first 13 weeks, and then every two weeks until 110 weeks of age. Body weight continued to be measured every 8 weeks until the end of the experiment. Status and behaviour of the animals were examined 3 times daily, and they were clinically examined for gross changes every 2 weeks. All animals were kept under observation until spontaneous death.

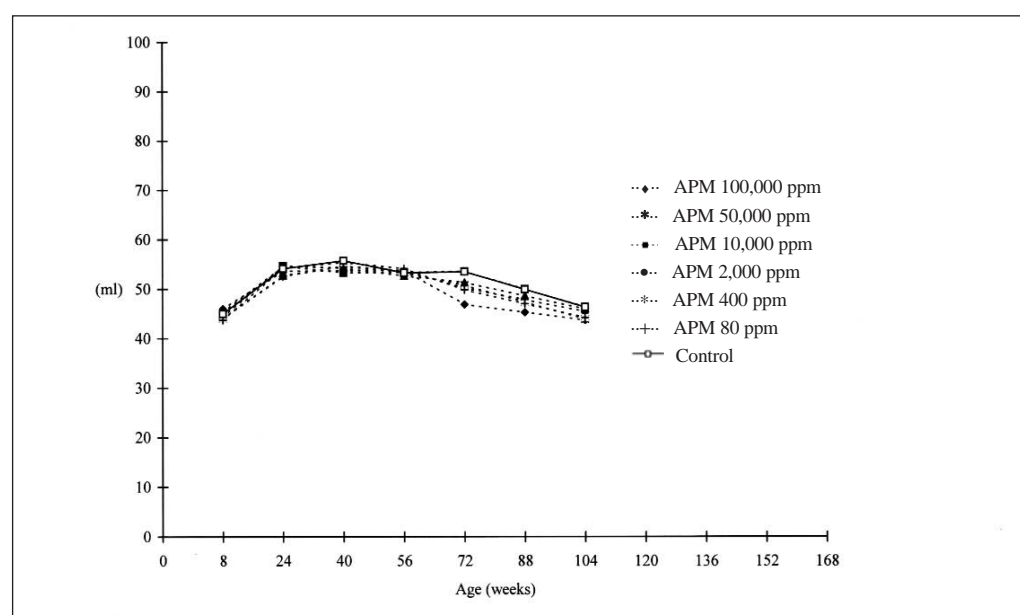


Fig. 1. Mean daily water consumption in male Sprague-Dawley rats

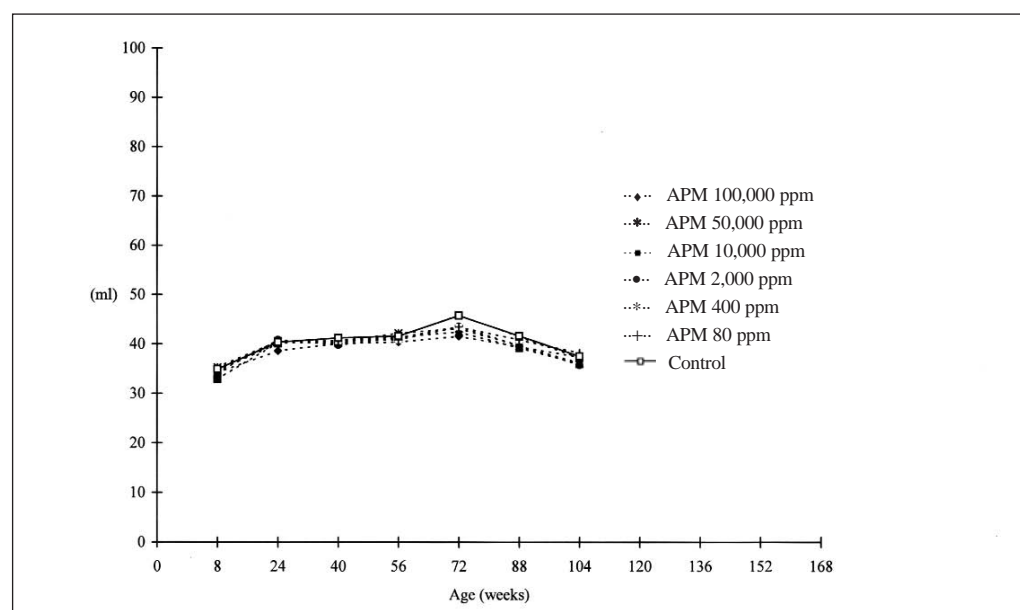


Fig. 2. Mean daily water consumption in female Sprague-Dawley rats

The biophase of the experiment terminated after 151 weeks, with the death of the last animal at the age of 159 weeks.

Upon death, the animals underwent complete necropsy.

Histopathology was routinely performed on the following organs and tissues of all animals from each group: skin and subcutaneous tissue, mammary gland, the brain (3 sagittal sections), pituitary gland, Zymbal glands, salivary glands, Harderian glands, cranium (five sections, with oral and nasal cavities and external and internal ear ducts), tongue, thyroid, parathyroid, pharynx, larynx, thymus and mediastinal lymph nodes, trachea, lung and mainstem bronchi, heart, diaphragm, liver, spleen, pan-

creas, kidneys, adrenal glands, oesophagus, stomach (fore and glandular), intestine (four levels), urinary bladder, prostate, gonads, interscapular brown fat pad, subcutaneous and mesenteric lymph nodes and other organs or tissues with pathological lesions.

All organs and tissues were preserved in 70% ethyl alcohol, except for bones which were fixed in 10% formalin and then decalcified with 10% formaldehyde and 20% formic acid in water solution. The normal specimens were trimmed, following the Standard Operating Procedures at the CRC laboratory: i.e. parenchymal organs were dissected through the hilus to expose the widest surface, and hollow organs were sectioned across the greatest diameter.

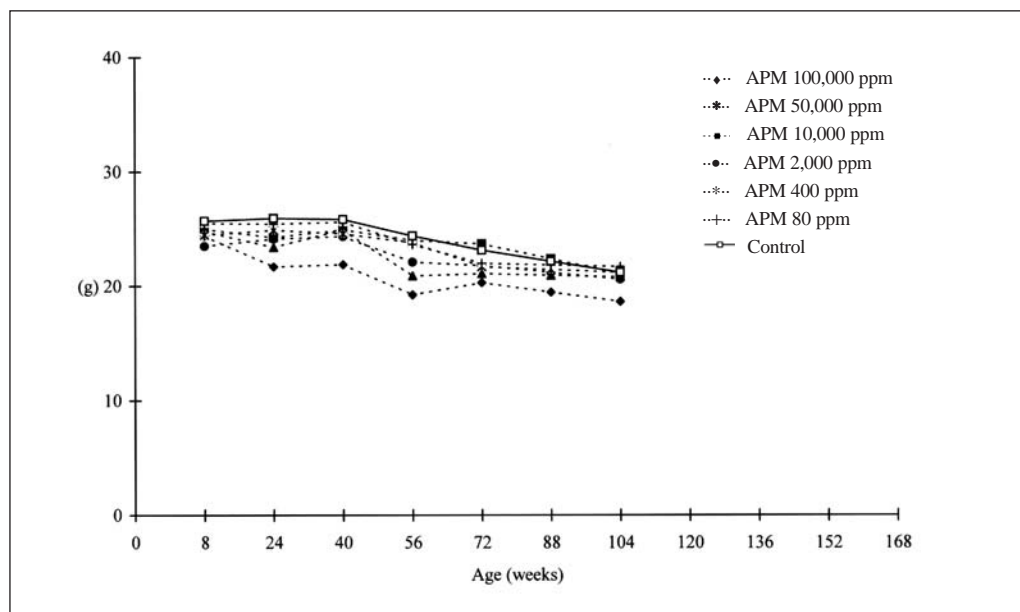


Fig. 3. Mean daily feed consumption in male Sprague-Dawley rats

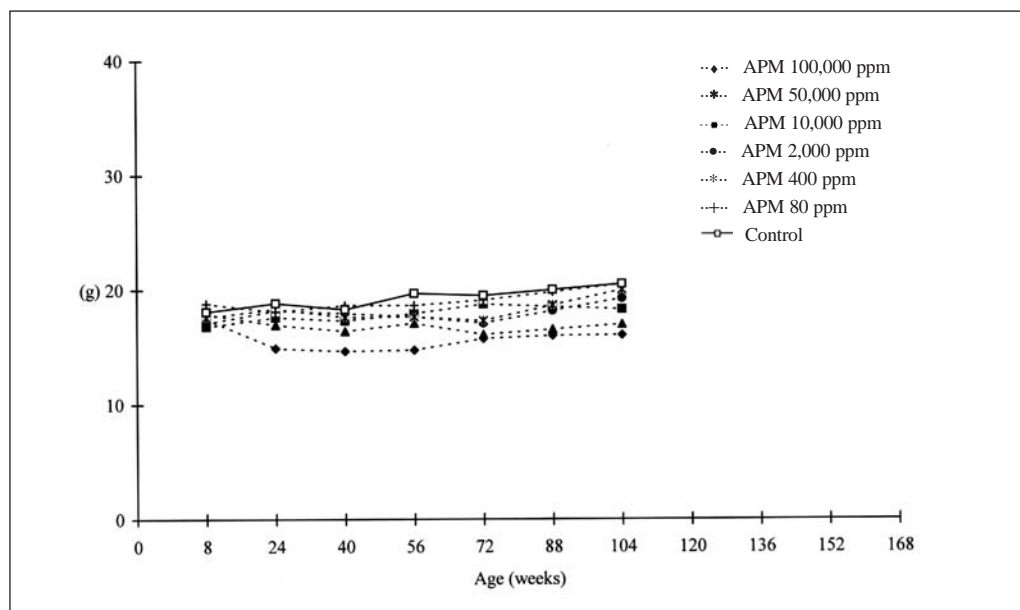


Fig. 4. Mean daily feed consumption in female Sprague-Dawley rats

Any pathological tissue was trimmed through the largest surface, including normal adjacent tissue. Trimmed specimens were processed as paraffin blocks, and 3-5 micron sections of every specimen were obtained. Sections were routinely stained with haematoxylin-eosin.

Statistical analyses were performed using the poly-k test (k = 3). This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account⁶⁻⁸.

Results

During the experiment no differences were observed among the various groups in mean daily water consump-

tion (figs. 1 and 2). A dose-related difference in feed consumption was observed between the various treated groups and the control group in both males and females (figs. 3 and 4). No differences in mean body weight were observed among treated and control groups in either males or females (figs. 5 and 6). No substantial difference in survival was observed among treated and control groups, males or females (figs. 7 and 8).

Yellowing of the coat was observed in animals exposed to APM, mainly at the highest concentrations. This change was previously observed in our laboratory in rats exposed to formaldehyde administered with drinking water⁹.

The occurrence of lymphomas and leukaemias among male and female rats in treated and control groups is

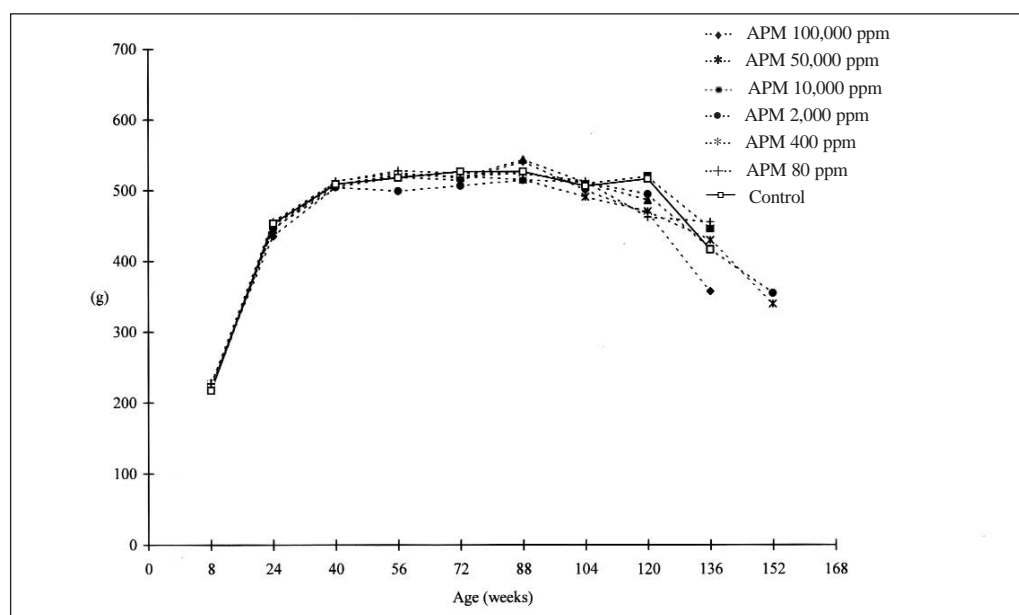


Fig. 5. Mean body weights in male Sprague-Dawley rats

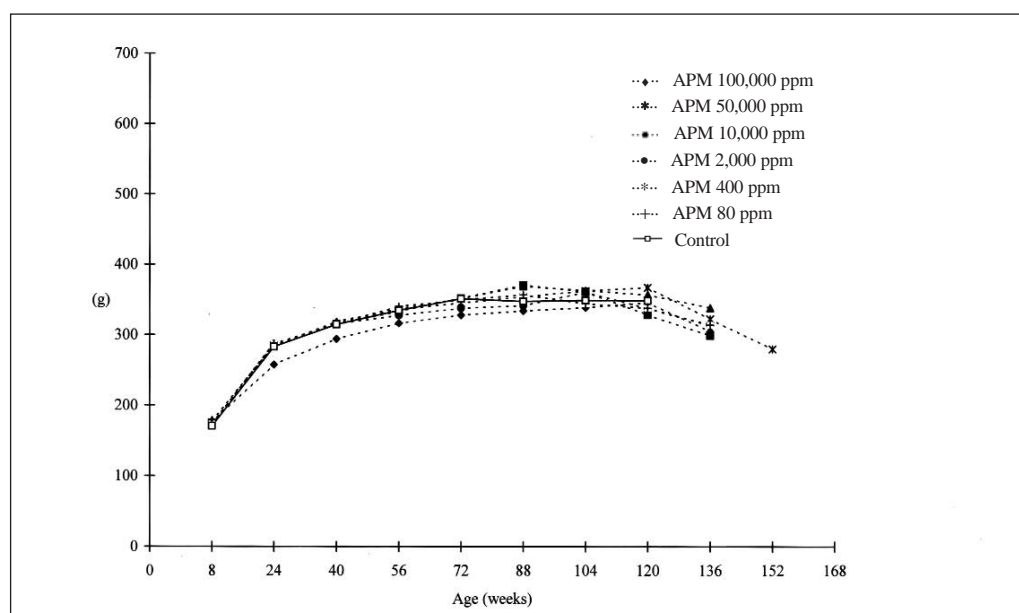


Fig. 6. Mean body weights in female Sprague-Dawley rats

shown in Table 2. The data indicate that APM causes a statistically significant increase in the incidence of lymphomas and leukaemias in females, at concentrations of 100,000 ($p \leq 0.01$); 50,000 ($p \leq 0.01$); 10,000 ($p \leq 0.05$); 2,000 ($p \leq 0.05$) and 400 ($p \leq 0.01$) ppm as compared to untreated controls. This increase is dose-related ($p \leq 0.05$).

Although not statistically significant, an increase was also observed in females treated with 80 ppm and in males treated with the highest dose.

The haemolymphoreticular neoplasias observed in the experiment include: lymphoblastic lymphoma and

leukaemia, lymphocytic lymphoma, lymphoimmunoblastic lymphoma, histiocytic sarcoma and monocytic leukaemia, myeloid leukaemia. The most frequent type of neoplasia was the lymphoimmunoblastic lymphoma (figs. 9 and 10).

Lymphomas and leukaemias are considered together, since both solid and circulating phases are present in many lymphoid neoplasms, and distinction between them is artificial¹⁰.

The occurrence of brain malignancies is shown in Table 3. Sparse malignant brain tumours were observed among males and females in the treated groups and none in the controls.

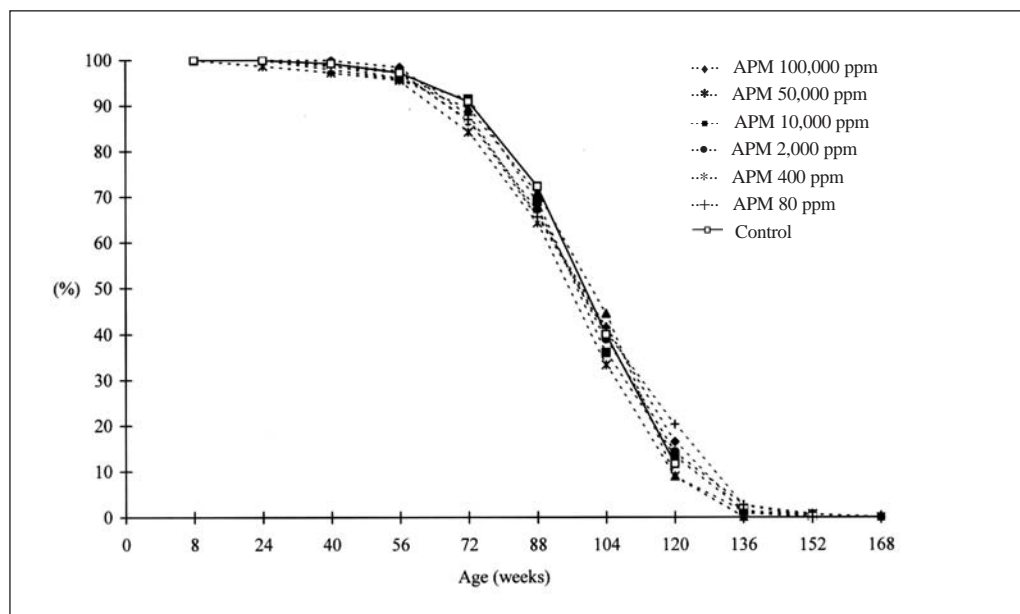


Fig. 7. Survival in male Sprague-Dawley rats

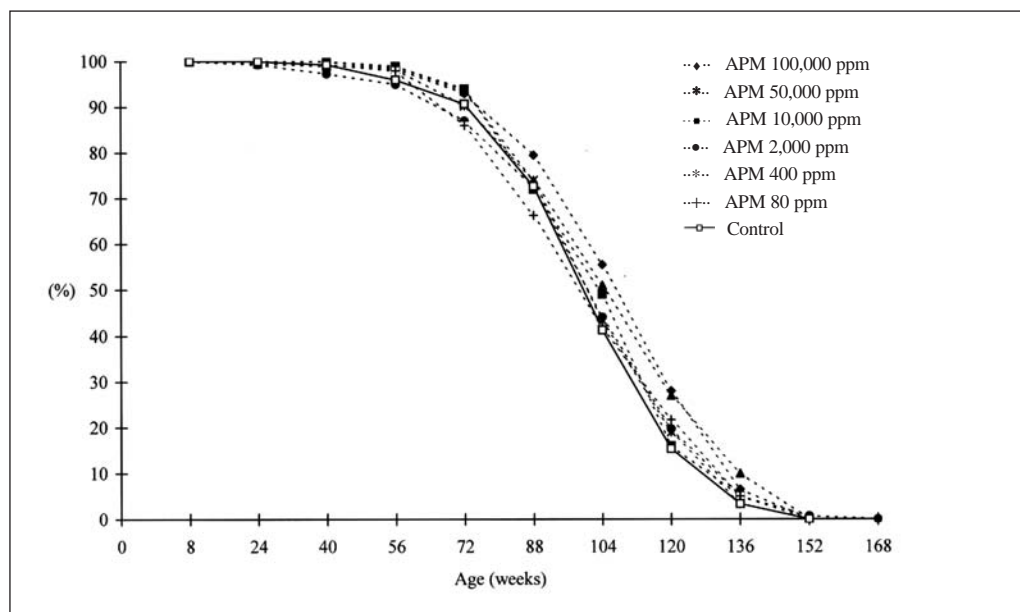


Fig. 8. Survival in female Sprague-Dawley rats

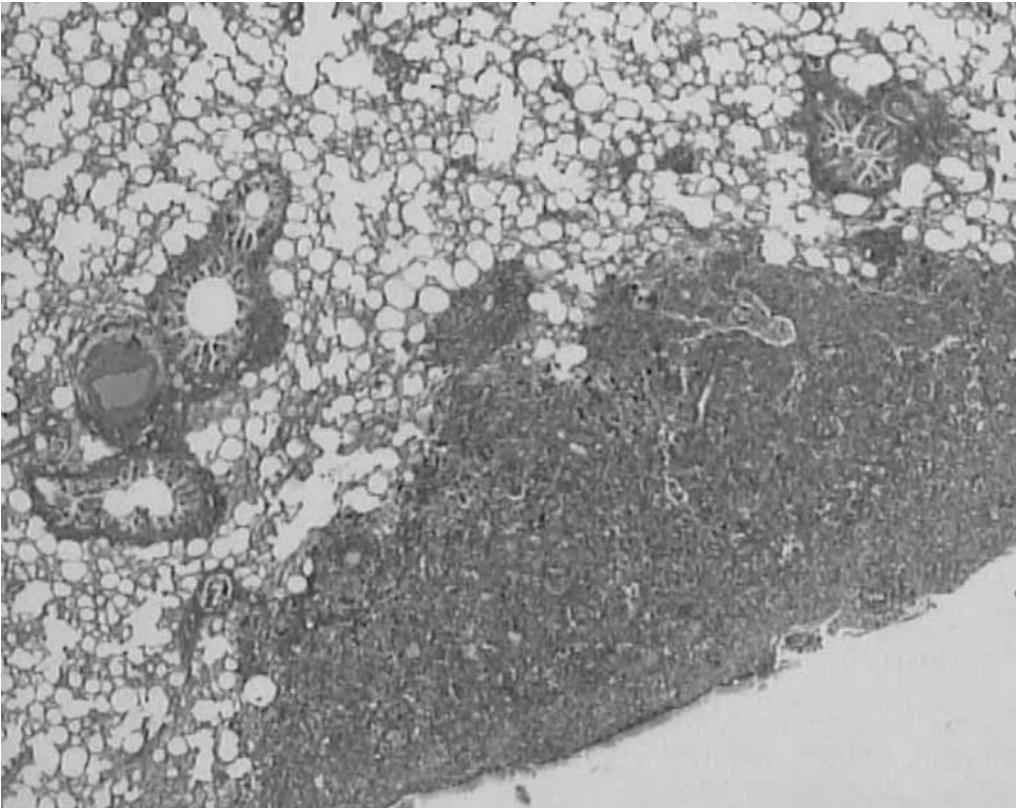


Fig. 9. Lymphoimmunoblastic lymphoma in a female rat administered 80 ppm aspartame in feed (lung). HE X 25

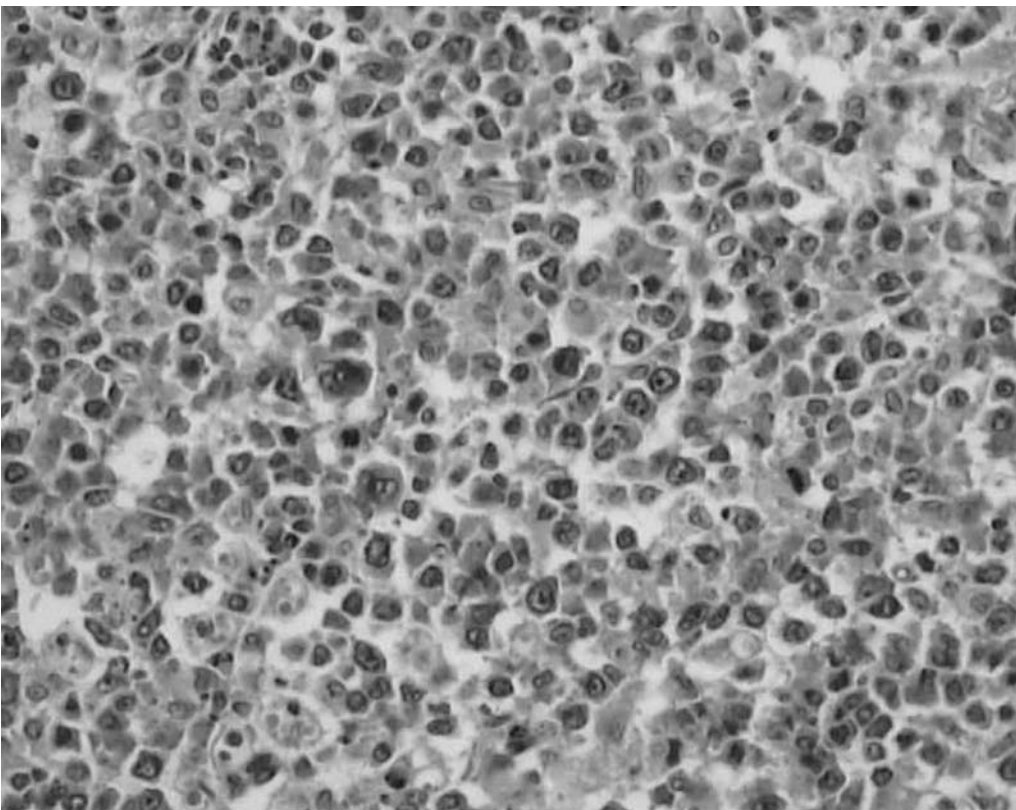


Fig. 10. A detail of the lymphoimmunoblastic lymphoma shown in fig. 9. HE X 400

Table 2 - Long-term carcinogenicity bioassay on aspartame administered with feed supplied *ad libitum* to male (M) and female (F) Sprague-Dawley rats from 8 weeks of age until spontaneous death. Incidence of lymphomas and leukaemias

Group No.	Animals			Treatment			Animals with lymphomas and leukaemias		
	Age at start (weeks)	Sex	No.	Dose			Duration	No.	%
				ppm	mg/kg b.w. ^a	Human ADI equivalent ^b			
I	8	M	100	100,000	5,000	100X	Life span	29	29.0
		F	100					25	25.0**
		M+F	200					54	27.0
II	8	M	100	50,000	2,500	50X	Life span	20	20.0
		F	100					25	25.0**
		M+F	200					45	22.5
III	8	M	100	10,000	500	10X	Life span	15	15.0
		F	100					19	19.0*
		M+F	200					34	17.0
IV	8	M	150	2,000	100	2X	Life span	33	22.0
		F	150					28	18.7*
		M+F	300					61	20.3
V	8	M	150	400	20	0.4X	Life span	25	16.7
		F	150					30	20.0**
		M+F	300					55	18.3
VI	8	M	150	80	4	0.08X	Life span	23	15.3
		F	150					22	14.7
		M+F	300					45	15.0
VII	8	M	150	0	-	-	Life span	31	20.7
		F	150					13	8.7
		M+F	300					44	14.7

^a Considering the life-span average weight of a rat (male and female) as 400 g and the average consumption of food as 20 g per day

^b Considering the Acceptable Daily Intake (ADI) of 50 mg/kg b.w. for humans

* Statistically significant $p \leq 0.05$; ** Statistically significant $p \leq 0.01$ using poly-k test ($k = 3$)

In our historical controls over the last 20 years, when we consider groups of 100 or more animals per sex (1934 males and 1945 females), the overall incidence of lymphomas and leukaemias in males is 20.7% (8.0-30.9) and in females 12.4% (7.0-18.4). The overall incidence of malignant brain tumours is 1.7% (0-5.0) in males and 0.7% (0-3.0) in females respectively.

Conclusions

In our experimental conditions, it has been demonstrated, for the first time, that APM causes a statistically significant, dose-related increase in lymphomas and leukaemias in females at dose levels very near those to which humans can be exposed. Moreover, it can hardly be overlooked that, at the lowest exposure of 80 ppm,

there was a 69% increase in lymphomas and leukaemias compared to controls, even though this was not statistically significant. When compared to the concurrent control group, an increase in the incidence of these neoplasias was also observed in males exposed to the highest dose; even though not statistically significant, this observation confirms and extends the result in females.

The significance of the increase in haemolymphoreticular neoplasias is further reinforced by the following considerations, based on the results of experiments performed in the CRC laboratory.

These experiments demonstrate that the increase in lymphomas and leukaemias, observed in the APM study, could be related to methanol, a metabolite of APM, which is metabolised to formaldehyde and then to formic acid, both in humans and rats³. In fact we have shown that: 1) methanol administered in drinking water increased the in-

Table 3 - Long-term carcinogenicity bioassay on aspartame administered with feed supplied *ad libitum* to male (M) and female (F) Sprague-Dawley rats from 8 weeks of age until spontaneous death. Incidence of malignant brain tumours

Group No.	Animals			Treatment			Animals with malignant brain tumours ^a		
	Age at start (weeks)	Sex	No.	Dose		Duration	No.	%	
				ppm	mg/kg b.w. ^b				Human ADI equivalent ^c
I	8	M	100	100,000	5,000	100X	Life span	1	1.0
		F	100					1	1.0
		M+F	200					2	1.0
II	8	M	100	50,000	2,500	50X	Life span	2	2.0
		F	100					1	1.0
		M+F	200					3	1.5
III	8	M	100	10,000	500	10X	Life span	0	-
		F	100					1	1.0
		M+F	200					1	0.5
IV	8	M	150	2,000	100	2X	Life span	2	1.3
		F	150					1	0.7
		M+F	300					3	1.0
V	8	M	150	400	20	0.4X	Life span	0	-
		F	150					0	-
		M+F	300					0	-
VI	8	M	150	80	4	0.08X	Life span	2	1.3
		F	150					1	0.7
		M+F	300					3	1.0
VII	8	M	150	0	-	-	Life span	0	-
		F	150					0	-
		M+F	300					0	-

^a The malignancies observed were: 10 malignant gliomas or mixed gliomas, 1 medulloblastoma, and 1 malignant meningioma

^b Considering the life-span average weight of a rat (male and female) as 400 g and the average consumption of food as 20 g per day

^c Considering the Acceptable Daily Intake (ADI) of 50 mg/kg b.w. for humans

cidence of lymphomas and leukaemias in female rats¹¹; 2) the same effect was induced in females treated with the gasoline oxygenated additive methyl-*tert*-butyl-ether (MTBE), which is also metabolised to methanol¹²; and finally 3) an increase in the incidence of lymphomas and leukaemias was also observed in females treated with formaldehyde^{9,13}.

These results further highlight the important rôle that formaldehyde has on the induction of haematological malignancies in rodents. Moreover, in a recent reevaluation of the carcinogenicity of formaldehyde by the International Agency for Research on Cancer (IARC), strong, although not considered sufficient, evidence of an association with leukaemias in humans was found¹⁴.

Since the results of carcinogenicity bioassays in rodents, mainly rats and mice, have been shown to be a consistent predictor of human cancer risk¹⁵⁻¹⁷, the first results of our

study call for urgent re-examination of permissible exposure levels of APM in both food and beverages, especially to protect children.

References

1. Butchko HH, Stargel WW, Comer CP, *et al.* Preclinical safety evaluation of aspartame. *Regul Toxicol Pharmacol* 2002; 35: S7-S16.
2. Aspartame Information Center. Available on <http://www.aspartame.org>, 2004.
3. Ranney RE, Opperman JA, Maldoon E, *et al.* Comparative metabolism of aspartame in experimental animals and humans. *Toxicol Environ Health* 1976; 2: 441-51.
4. Food and Drug Administration. Aspartame: Commissioner's Final Decision; 1981 *Fed Regist* 46, 38285-308.
5. Repubblica Italiana. Decreto Legislativo 116. Attuazione della direttiva n. 86/609/CEE in materia di protezione degli animali

- utilizzati a fini sperimentali o ad altri fini scientifici. Supplemento ordinario alla Gazzetta Ufficiale 1992; 40: 5-25.
6. Bailer AJ, Portier CJ. Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples. *Biometrics* 1988; 44: 417-31.
 7. Portier CJ, Bailer AJ. Testing for increased carcinogenicity using a survival-adjusted quantal response test. *Fundam Appl Toxicol* 1989; 12: 731-7.
 8. Piergorsh WW, Bailer AJ. *Statistics for environmental biology and toxicology*. London: Chapman, 1997.
 9. Soffritti M, Belpoggi F, Lambertini L, et al. Results of long-term experimental studies on the carcinogenicity of formaldehyde and acetaldehyde in rats. In Mehlman MA, Bingham E, Landrigan PJ, et al. *Carcinogenesis bioassays and protecting public health. Commemorating the lifework of Cesare Maltoni and colleagues*. *Ann NY Acad Sci* 2002; 982: 87-105.
 10. Harris NL, Jaffe ES, Vardiman JW, et al. WHO classification of tumours of haematopoietic and lymphoid tissues. Introduction. In Jaffe ES, Harris NL, Stein H, et al. *Tumours of haematopoietic and lymphoid tissues*. Lyon: IARC Press, 2001, 12-3.
 11. Soffritti M, Belpoggi F, Cevolani D, et al. Results of long-term experimental studies on the carcinogenicity of methyl alcohol and ethyl alcohol in rats. In Mehlman MA, Bingham E, Landrigan PJ, et al. *Carcinogenesis bioassays and protecting public health. Commemorating the lifework of Cesare Maltoni and colleagues*. *Ann NY Acad Sci* 2002; 982: 46-69.
 12. Belpoggi F, Soffritti M, Maltoni C. Methyl-tertiary-butyl ether (MTBE), a gasoline additive, causes testicular and lymphohaematopoietic cancers in rats. *Toxicol Ind Health* 1995; 11: 119-49.
 13. Soffritti M, Maltoni C, Maffei F, et al. Formaldehyde: an experimental multipotent carcinogen. *Toxicol Ind Health* 1989; 5: 699-730.
 14. International Agency for Research on Cancer. *Monographs on the evaluation of the carcinogenic risk of chemicals to humans. Formaldehyde, 2-Butoxyethanol and 1-tert-Butoxy-2-Propanol*. Vol. 88 (in press). Available on <http://www.iarc.fr>.
 15. Huff J. Long-term chemical carcinogenesis bioassays predict human cancer hazards. Issues, controversies, and uncertainties. In Bailer JA, Maltoni C, Bailar III JC, et al. *Uncertainty in the risk assessment of environmental and occupational hazards*. *Ann NY Acad Sci* 1999; 895: 56-79.
 16. Tomatis L, Aitio A, Wilbourn J, et al. Human carcinogens so far identified. *Jpn J Cancer Res* 1989; 80: 795-807.
 17. Rall DP. Can laboratory animal carcinogenicity studies predict cancer in exposed children? *Environ Health Perspect* 1995; 103 suppl 6: 173-5.

アスパルテームはラットにおいてリンパ腫と白血病を誘発する

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要約

広く使用されている人口甘味料であるアスパルテームの 100,000、50,000、10,000、2,000、400、80 および 0 ppm を 8 週齢の Sprague-Dawley 系雌雄ラット（各群雌雄それぞれ 100-150 匹）に飼料とともに投与した。この投与は動物の自然死まで継続した。その結果、我々はアスパルテームの投与に関連した統計的に有意な雌動物のリンパ腫と白血病誘発についての最初の結果をここに報告する。また、対照と比較して統計的に有意ではなかったが、悪性脳腫瘍が投与群の動物に観察された。

キーワード：アスパルテーム、人工甘味料、発癌性、ラット、リンパ腫、白血病

緒言

アスパルテーム (APM) は、世界中で何億人という人達に広く使用されている甘味料である^{1,2}。これは、ソフトドリンク、チューインガム、キャンデー、ヨーグルト、食卓用甘味料およびビタミンや無糖の咳止めドロップなどいくつかの薬品など 6000 品目を越える製品に含まれている²。

APM の消費者について行った食事に関する調査により、APM の一日平均摂取量は、一般の人達では体重に対し 2 から 3 mg/kg であり、その量は子供や妊娠婦人では一層多いものであった¹。米国およびヨーロッパにおいては、一日摂取許容量 (ADI) はそれぞれ体重に対し 50 および 40 mg/kg である¹。

げっ歯類動物およびヒトにおいては、APM は消化管において、アスパラギン酸、フェニルアラニンおよびメタノールの 3 種類の成分に代謝される³。

1970 年代に APM に関する長期混餌投与と発癌性試験が、ラットを用いて 3 回、マウスを用いて 1 回実施された。全体的に、発癌性試験は陰性であると考えられたが、これらの試験は、現在では化学物質や物理的要因の発癌性試験時に満たさなければならない基本的要件を順守していなかった点に留意しなければならない。こうした限界があるため、我々は現在承認されている GLP (医薬品安全性試験実施基準) にしたがって大々的な実験を実施

することとした。

この論文において、我々はその実験で始めて得た血液リンパ網内系悪性腫瘍（リンパ腫および白血病）および悪性脳腫瘍についての結果を報告する。