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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), 00-00, 2005

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

Riassunto

L'aspartame, un dolcificante artificiale largamente diffuso, è stato somministrato con il mangime a ratti Sprague-Dawley, maschi e femmine (100-150/sesso/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), 00-00, 2005

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

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-top sweeteners and some pharmaceuticals such as vitamins and sugar-free cough drops[2].

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 women[1]. 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., respectively[1].

In rodents and humans, APM is metabolised in the gastrointestinal tract into three constituents: aspartic acid, phenylalanine and methanol[3].

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

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. The animals were kept in one single room, at 23 ± 2°C and 50-60% relative humidity.

Once a week for the first 13 weeks, then every two weeks until 110 weeks of age, the mean daily drinking water and feed consumption were measured per cage, and body weight individually. 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.

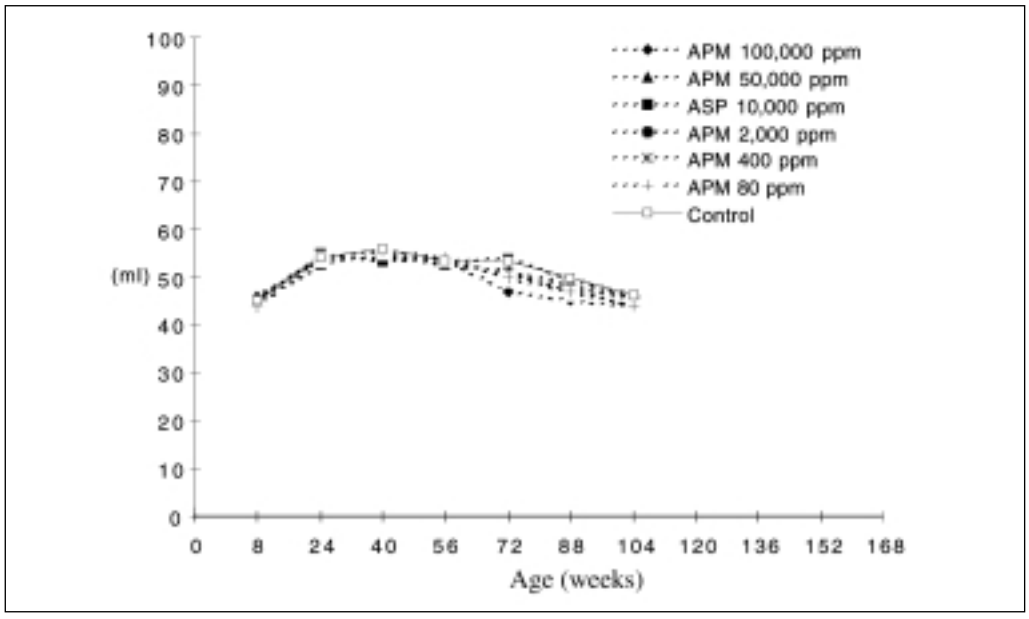

Fig. 1. Mean daily water consumption in male Sprague-Dawley rats

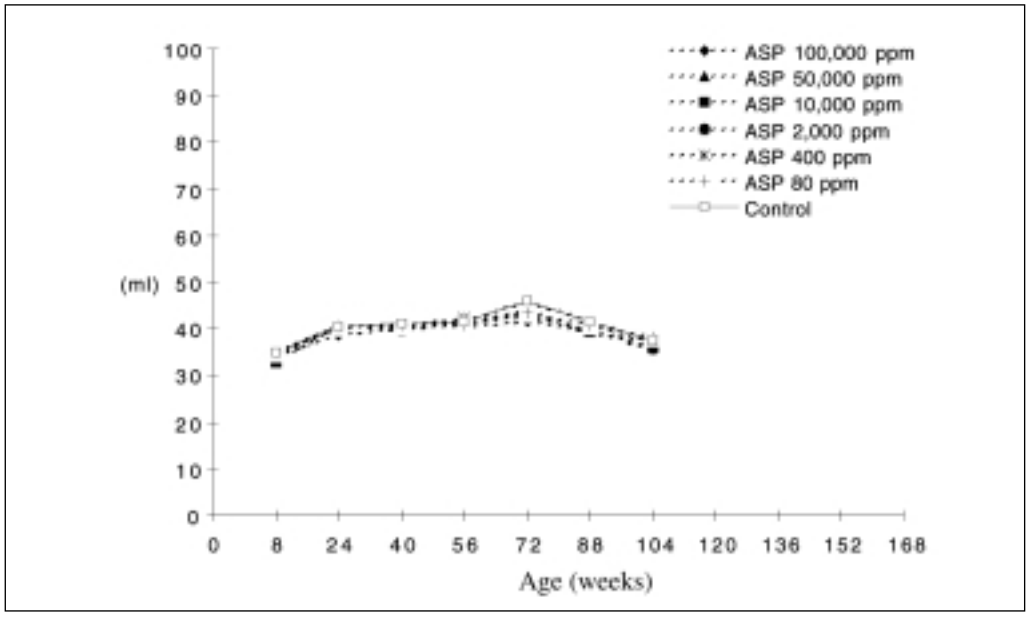

Fig. 2. Mean daily water consumption in female Sprague-Dawley rats

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, pancreas, 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.

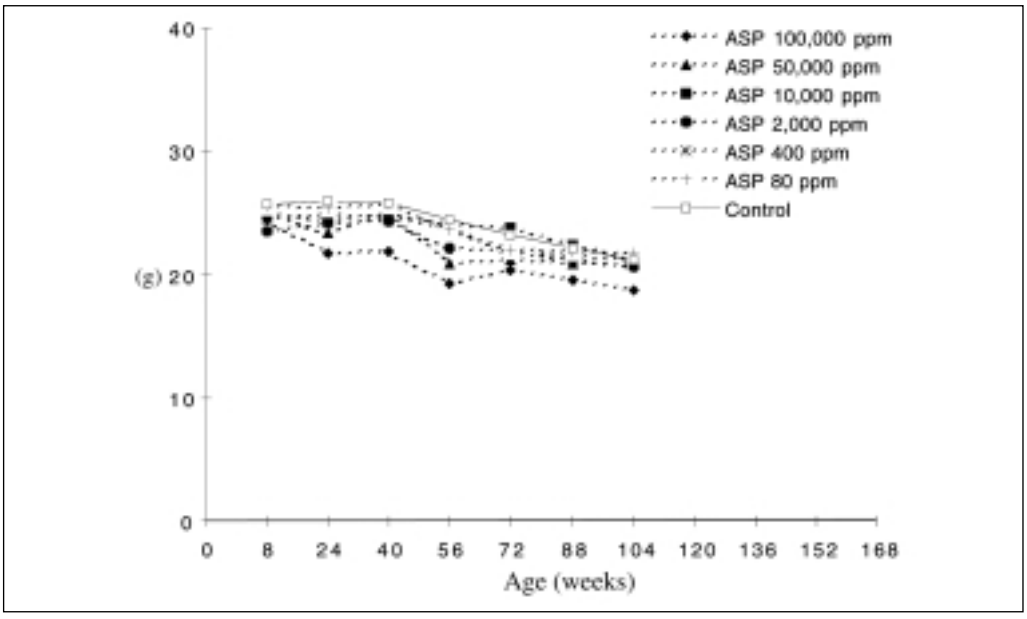

Fig. 3. Mean daily feed consumption in male Sprague-Dawley rats

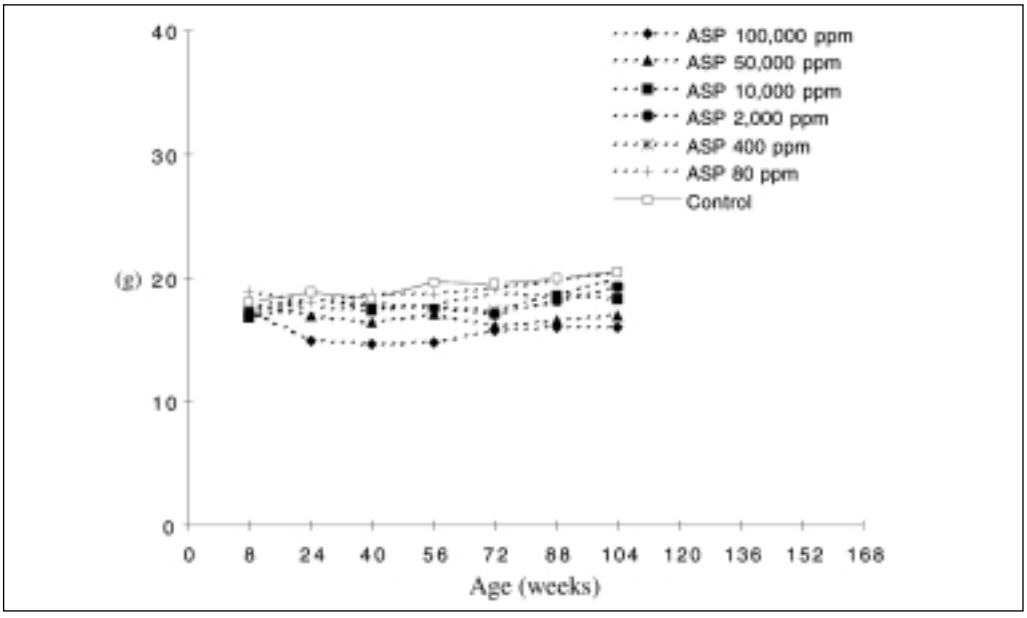

Fig. 4. Mean daily feed consumption in female Sprague-Dawley rats

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 account[6-8].

Results

During the experiment no differences were observed among the various groups in mean daily water consumption (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 water[9].

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

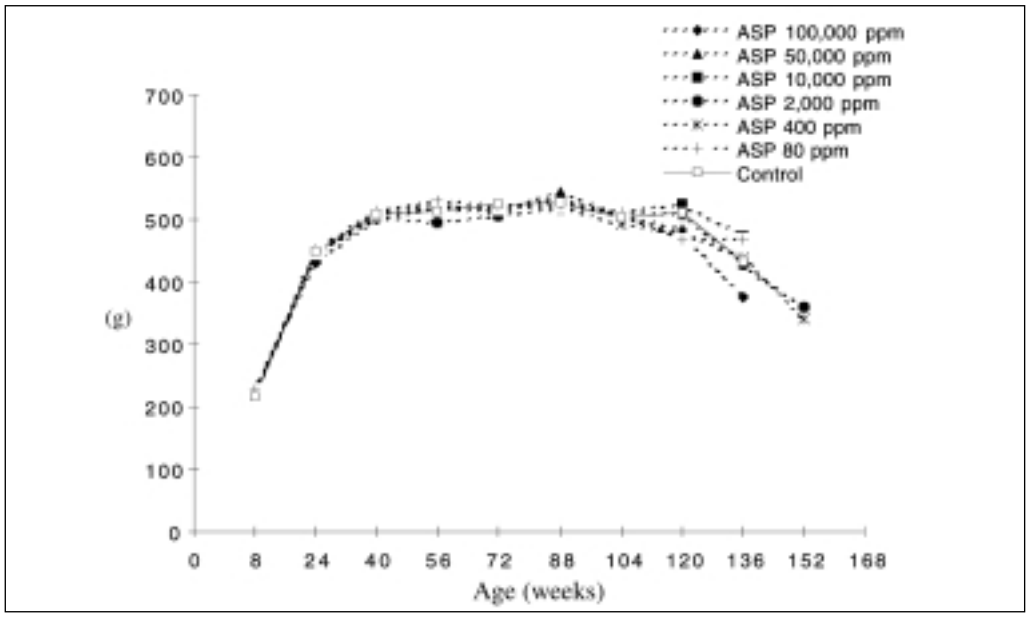

Fig. 5. Mean body weights in male Sprague-Dawley rats

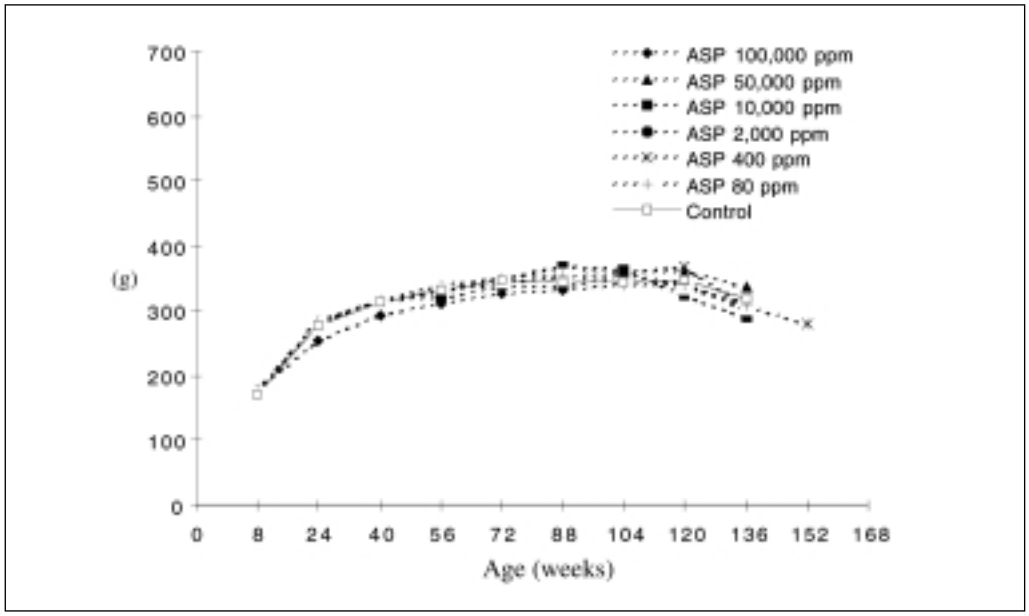

Fig. 6. Mean body weights in female Sprague-Dawley rats

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 ($p \leq 0.01$) 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 artificial[10].

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.

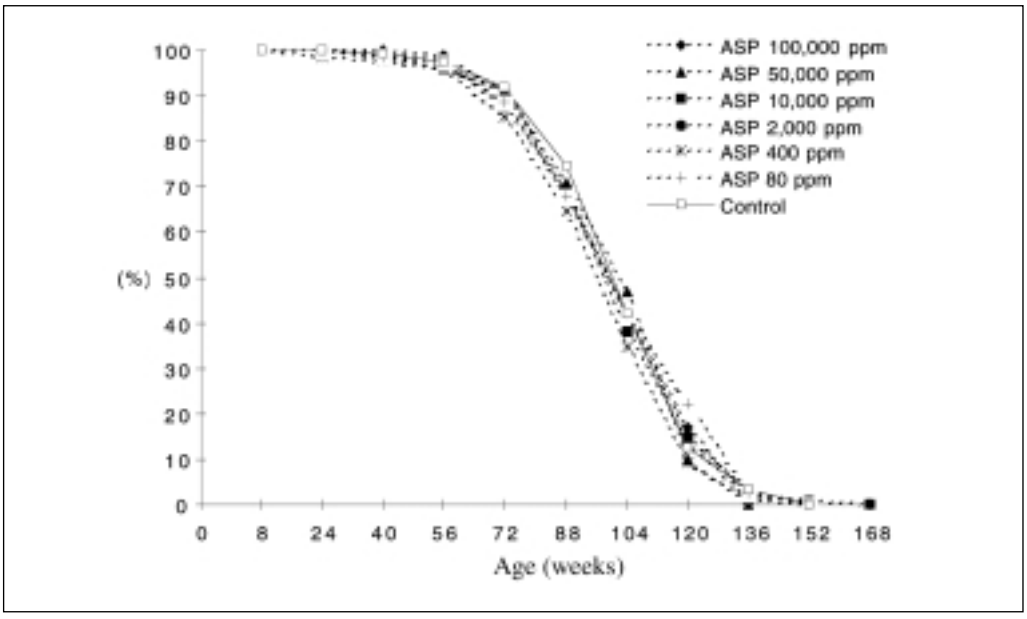

Fig. 7. Survival in male Sprague-Dawley rats

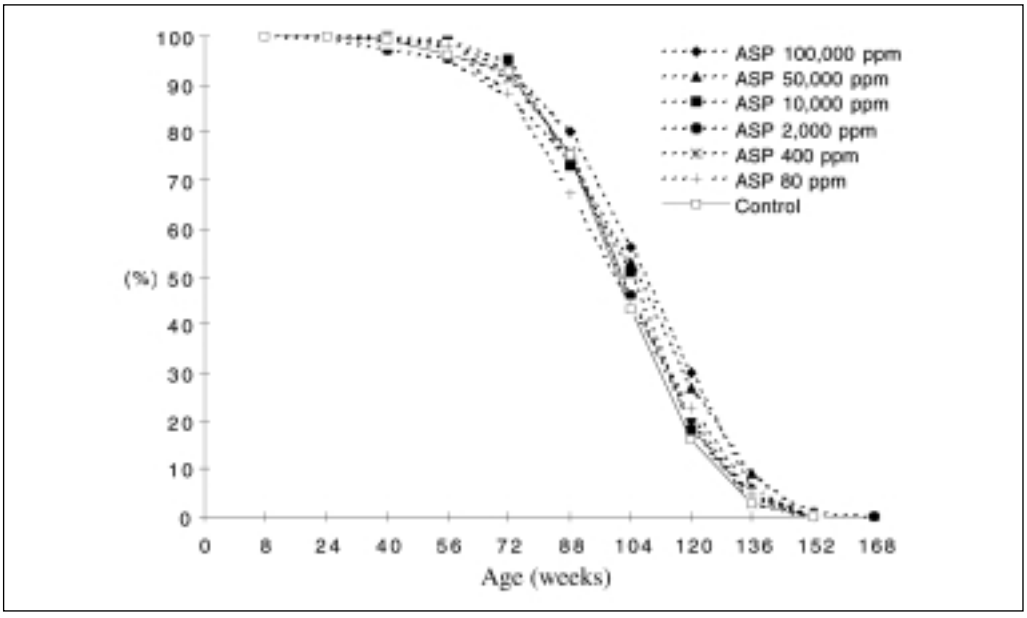

Fig. 8. Survival in female Sprague-Dawley rats

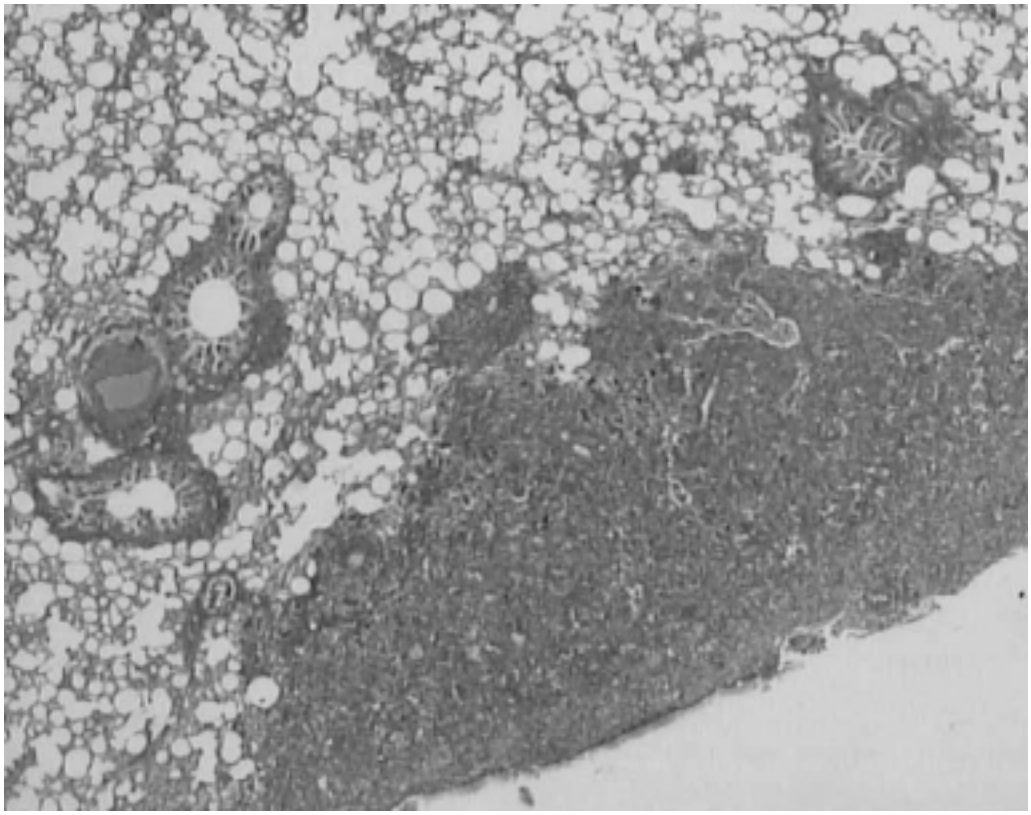

Fig. 9. Lymphoimmunoblastic lymphoma in a female rat administered 80 ppm aspartame in feed (lung). HE X 25

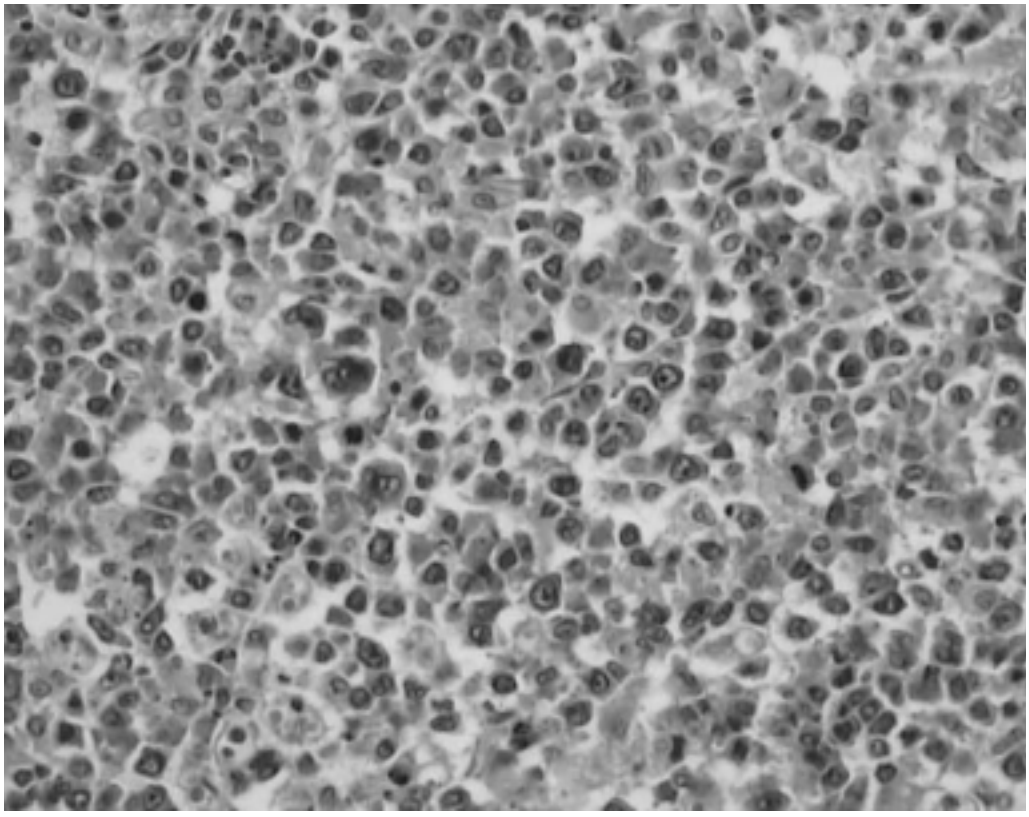

Fig. 10. A detail of the lymphoimmunoblastic lymphoma shown in fig. 9. HE X 400

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					29	29.0
		F	100	100,000	5,000	100X	Life span	25	25.0**
		M+F	200					54	27.0
II	8	M	100					20	20.0
		F	100	50,000	2,500	50X	Life span	25	25.0**
		M+F	200					45	22.5
III	8	M	100					15	15.0
		F	100	10,000	500	10X	Life span	19	19.0*
		M+F	200					34	17.0
IV	8	M	150					33	22.0
		F	150	2,000	100	2X	Life span	28	18.7*
		M+F	300					61	20.3
V	8	M	150					25	16.7
		F	150	400	20	0.4X	Life span	30	20.0**
		M+F	300					55	18.3
VI	8	M	150					23	15.3
		F	150	80	4	0.08X	Life span	22	14.7
		M+F	300					45	15.0
VII	8	M	150					31	20.7
		F	150	0	-	-	Life span	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 1957 females), the overall incidence of lymphomas and leukaemias in males is 21.8% (8.0-30.9) and in females 13.4% (7.0-18.4). The overall incidence of malignant brain tumours is 1.7% (0-5.0) in males and 0.7% (0-2.0) in females respectively.

Conclusions

In our experimental conditions, it has been demonstrated, for the first time, that APM causes a dose-related statistically significant 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 62% 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 rats[3]. 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					1	1.0
		F	100	100,000	5,000	100X	Life span	1	1.0
		M+F	200					2	1.0
II	8	M	100					2	2.0
		F	100	50,000	2,500	50X	Life span	1	1.0
		M+F	200					3	1.5
III	8	M	100					0	-
		F	100	10,000	500	10X	Life span	1	1.0
		M+F	200					1	0.5
IV	8	M	150					2	1.3
		F	150	2,000	100	2X	Life span	1	0.7
		M+F	300					3	1.0
V	8	M	150					0	-
		F	150	400	20	0.4X	Life span	0	-
		M+F	300					0	-
VI	8	M	150					2	1.3
		F	150	80	4	0.08X	Life span	1	0.7
		M+F	300					3	1.0
VII	8	M	150					0	-
		F	150	0	-	-	Life span	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 rats[11]; 2) the same effect was induced in females treated with the gasoline oxygenated additive methyl-*tert*-butyl-ether (MTBE), which is also metabolised to methanol[12]; 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 found[14].

Since the results of carcinogenicity bioassays in rodents, mainly rats and mice, have been shown to be a consistent predictor of human cancer risk[15-17], 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.

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