

Results of Long-Term Carcinogenicity Bioassay on Sprague-Dawley Rats Exposed to Aspartame Administered in Feed

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The end judges everything
—HERODOTUS (480-425 B.C.)
The History

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ABSTRACT: Aspartame (APM) is one of the most widely used artificial sweeteners in the world. Its ever-growing use in more than 6000 products, such as soft drinks, chewing gum, candy, desserts, etc., has been accompanied by rising consumer concerns regarding its safety, in particular its potential long-term carcinogenic effects. In light of the inadequacy of the carcinogenicity bioassays performed in the 1970s and 1980s, a long-term mega-experiment on APM was undertaken at the Cesare Maltoni Cancer Research Center of the European Ramazzini Foundation on groups of male and female Sprague-Dawley rats (100–150/sex/group), 8 weeks old at the start of the experiment. APM was administered in feed at concentrations of 100,000, 50,000, 10,000, 2,000, 400, 80, or 0 ppm. Treatment lasted until spontaneous death of the animals. The results of the study demonstrate that APM causes: (a) an increased incidence of malignant tumor-bearing animals, with a positive significant trend in both sexes, and in particular in females treated at 50,000 ppm ($P \leq 0.01$) when compared to controls; (b) an increase in lymphomas–leukemias, with a positive significant trend in both sexes, and in particular in females treated at doses of 100,000 ($P \leq 0.01$), 50,000 ($P \leq 0.01$), 10,000 ($P \leq 0.05$), 2000 ($P \leq 0.05$), and 400 ppm ($P \leq 0.01$); (c) a statistically significant increased incidence, with a positive significant trend, of transitional cell carcinomas of the renal pelvis and ureter in females and particularly in those treated at 100,000 ppm ($P \leq 0.05$); and (d) an increased incidence of malignant

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schwannomas of the peripheral nerves, with a positive trend in males ($P \leq 0.05$). The results of this mega-experiment indicate that APM, in the tested experimental conditions, is a multipotential carcinogenic agent.

KEYWORDS: aspartame; carcinogenicity; long-term bioassays; rat

INTRODUCTION

The introduction of artificial sweeteners as substitutes for sucrose began during World Wars I and II, when the use of saccharin became prevalent due to its low cost and the wartime shortage of table sugar.¹ In the following years, two additional artificial sweeteners were introduced to the market: cyclamate in the 1950s and aspartame (APM) in 1981. Since the 1970s, the growing obesity problem in industrialized countries, due in part to fast food and soft drink consumption, has led to an increased demand for reduced-calorie food-stuffs. Given the lucrative market for these so-called “diet” or “light” products, additional new-generation sweeteners have emerged, including acesulfame-K, sucralose, and neotame.²

With the expansion of the artificial sweetener market, concerns have arisen among consumers regarding the safety of these sweeteners and their possible long-term health effects. At the center of this debate has been the question of the potential carcinogenic risks associated with artificial sweetener use. Until now, no adequate epidemiological or experimental animal studies have been available.

Most epidemiological studies, aimed to evaluate the relationship between artificial sweetener intake and cancer, have focused on sweetener consumption in general, and not on single compounds.³ This limitation is attributed to the fact that most consumers use multiple artificial sweeteners, as different sweeteners are often blended together in food products. Moreover, given the fact that wide consumption of artificial sweeteners emerged in the 1980s and 1990s, epidemiological studies are, by definition, limited in terms of exposure to the compounds.

Most long-term carcinogenicity bioassays performed on rodents over the last 30 years have not been adequately designed to assess carcinogenic risk. The sensitivity of these studies in detecting risk has been greatly limited by the following factors: (a) the number of animals per sex per group was usually 50 or less; (b) the experiments were usually truncated at 104 weeks (or earlier) from the start of the experiment, thus not allowing the tested compound to express its carcinogenic potential; and (c) the conduct of the experiments was often inadequate with incomplete or nonsystematic histopathological analysis for all organs and tissues.

Because of the globalization of the industrialized diet and the ever-increasing use of artificial sweeteners among billions of people in both industrialized and

ranges from 2 to 3 mg/kg of body weight (h.w.) in the general population.¹⁰ These surveys also show that consumption by children and young women range from about 2.5 to 5 mg/kg b.w./day.¹⁰ The Acceptable Daily Intake (ADI) of APM in the United States is 50 mg/kg b.w. and in Europe is 40 mg/kg b.w.¹⁰

APM is metabolized in rodents, nonhuman primates, and humans in the gastrointestinal tract into three constituents (aspartic acid, phenylalanine, and methanol) which are then absorbed and enter into the systemic circulation.¹¹ After absorption, these compounds follow the same metabolic path as when ingested through other foods: aspartate and phenylalanine are used as amino acidic building blocks for protein synthesis or transformed, respectively, into alanine plus oxalacetate¹² and tyrosine (and, partially, into phenylethylamine and phenylpyruvate).¹³ Methanol is oxidized to formaldehyde and then to formic acid.¹⁴

APM has been tested for genotoxicity in both *in vivo* and *in vitro* tests. *In vitro*, an assay to measure the induction of unscheduled DNA synthesis in rat hepatocytes was reported to be negative, suggesting the absence of induced DNA damage by APM.¹⁵ APM was also evaluated *in vitro* in a chromosomal aberration test, a sister chromatid exchange (SCE) test, and in a micronuclei test on human lymphocytes.¹⁶ In the chromosomal aberration test, statistically significant increases (2.5-4.2-fold, compared to control values) in the percentage of aberrant cells or in the number of chromosomal aberrations per cell were observed in all doses. No effect of APM was observed in the SCE test. In the micronuclei test, a statistically increased incidence in cells with micronucleus was observed at the highest dose of treatment.

In vivo results of a test for the induction of chromosomal aberration in bone marrow cells of male Swiss mice, after the administration by gavage of a mixture of APM (up to 350 mg/kg) and acesulfame potassium (up to 150 mg/kg), were negative. A dose-related increase in the percentage of cells with chromosomal aberrations was noted with increasing doses of the two sweeteners; however, the increase was not statistically significant.¹⁷ In a peripheral blood micronuclei test conducted on p53 haploinsufficient mice exposed for 9 months to 50,000, 25,000, 12,500, 6250, 3125, or 0 ppm to APM in feed, the results were judged to be positive in females on the basis of a significant trend test and the increased frequency of micronucleated erythrocytes observed in the 50,000 ppm group.¹⁸

Epidemiological studies to evaluate the relationship between APM intake and the development of cancer in humans are not currently available, with the exception of one study in which an increased incidence of brain tumors in the United States between the 1970s and 1980s was linked to agents/situations of risk of environmental origin, and among them, consumption of APM.¹⁹

Four long-term experimental bioassays were performed on rodents in the 1970s and early 1980s. Two long-term feeding carcinogenicity bioassays on APM were performed on Sprague-Dawley rats and one on mice by Searle

& Co., the results of which were reviewed by the FDA and summarized in the Federal Register of 1981.²⁰ To date, the details of these experiments have not been published. A fourth experiment was performed on Wistar rats by Japanese researchers and the results published in 1981, without exhaustive experimental details.^{21,22} The results of these four experiments did not show any carcinogenic effects of APM in the tested experimental conditions. The study design, conduct, and results of these experiments were discussed by the ERF in a previous article.²³

In 2005, a carcinogenicity study on APM was performed by the U.S. National Toxicology Program on genetically altered strains of mice, namely p53 haploinsufficient, Tg AC hemizygous, and Cdkn2a deficient male and female mice which develop, with increased susceptibility and decreased latency, lymphomas or sarcomas, squamous cell papillomas/carcinomas of the forestomach and brain tumors, respectively.¹⁸ Feed containing 50,000, 25,000, 12,500, 6250, 3125, or 0 ppm was administered for 40 weeks to groups of 15 males and 15 females. Although the Technical Report states that in the tested experimental conditions, no evidence of carcinogenic effects was observed, the conclusions of the study also include the following qualification: "because this is a new model, there is uncertainty whether the study possessed sufficient sensitivity to detect a carcinogenic effect."¹⁸

In light of the inadequacies and uncertainties surrounding the available epidemiological and long-term experimental data on APM, the Cesare Maltoni Cancer Research Center (CMCRC)/ERF decided to perform a life-span mega-experiment which would evaluate the carcinogenic potential of APM when administered in feed to Sprague-Dawley rats.

MATERIALS AND METHODS

The APM used as a food grade material was produced by Nutrasweet and supplied by Giusto Faravelli S.p.A. in Milan, Italy. Its purity was >98%. The impurities included DKP <1.5% and L-phenylalanine <0.5%. The method used to determine its purity was an infrared absorption spectrophotometer assay. APM was added to the standard Corticella pellet diet, used for 30 years at the CMCRC/ERF Laboratory, at concentrations of 100,000, 50,000, 10,000, 2000, 400, 80, or 0 ppm, to simulate an assumed daily intake by humans of 5000, 2500, 500, 100, 20, 4, or 0 mg/kg b.w. The APM daily consumption in mg/kg b.w. for both males and females was calculated considering the average weight of a rat as 400 g for the duration of the experiment and the average consumption of feed as 20 g per day. APM was administered in feed *ad libitum* to Sprague-Dawley rats (100–150/sex/group), 8 weeks old at the start of the experiment. The treatment lasted until natural death. Control animals received the same feed without APM. Upon death, all animals underwent complete necropsy. The general protocols of the experiment, including methods of tumor reporting and

TABLE 1. Continued.

Site	Groups																											
	I: 100,000 ppm				II: 50,000 ppm				III: 10,000 ppm				IV: 2,000 ppm				V: 400 ppm				VI: 80 ppm				VII: 0 ppm (control)			
	Male	Female	No.	%	Male	Female	No.	%	Male	Female	No.	%	Male	Female	No.	%	Male	Female	No.	%	Male	Female	No.	%	Male	Female	No.	%
Nasal cavities ^b	0	2	2.0	1	1.0	0	-	2	2.0	2	2.0	1	0.7	1	0.7	0	-	1	0.7	1	0.7	2	1.3	0	-	0	-	
Adenoma	1	1.0	0	-	0	0	-	0	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	
Squamous cell carcinoma	0	1	1.0	0	-	0	0	-	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	
Olfactory neuroblastoma	0	-	1	1.0	0	-	0	0	-	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	
Oral cavity & lips	0	-	2	2.0	1	1.0	0	-	1	1.0	1	1.0	1	0.7	0	-	1	0.7	0	-	0	0	1	0.7	0	-	0	-
Acanthoma	1	1.0	2	2.0	0	-	1	1.0	0	-	1	1.0	0	-	1	1.0	0	-	1	1.0	0	-	0	0	0	0	0	0
Squamous cell carcinoma	0	-	0	0	-	0	0	-	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0
Pharynx	0	-	0	0	-	0	0	-	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0
Squamous cell carcinoma	0	-	0	0	-	0	0	-	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0
Lung	1	1.0	0	-	0	0	-	2	2.0	0	-	1	0.7	2	1.3	0	-	0	0	0	0	0	0	0	0	0	0	0
Adenoma	0	-	0	0	-	0	0	-	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0
Fibroma	0	-	0	0	-	1	1.0	0	-	0	0	0	0	0	0	-	1	0.7	0	-	0	0	0	0	0	0	0	0
Fibrosarcoma	0	-	0	0	-	1	1.0	0	-	0	0	0	0	0	0	-	2	1.3	0	-	0	0	0	0	0	0	0	0
Squamous cell carcinoma	0	-	0	0	-	1	1.0	0	-	0	0	0	0	0	0	-	1	0.7	1	0.7	0	-	0	0	0	0	0	0
Hemangiosarcoma	1	1.0	0	-	1	1.0	0	-	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0
Pleura	0	-	0	0	-	1	1.0	0	-	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0
Mesothelioma	0	-	0	0	-	0	0	-	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0
Stomach	0	-	0	0	-	0	0	-	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0
- Fore stomach	0	-	0	0	-	0	0	-	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0
Polyp	3	3.0	2	2.0	3	3.0	0	-	2	2.0	3	3.0	3	2.0	0	-	0	0	0	0	0	0	0	0	0	0	0	0
Acanthoma	0	-	0	0	-	1	1.0	0	-	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0
Squamous cell carcinoma	0	-	0	0	-	1	1.0	0	-	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0
- Glandular	0	-	0	0	-	0	0	-	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0
Polyp	0	-	0	0	-	0	0	-	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0
Leiomyosarcoma	0	-	1	1.0	0	-	0	0	-	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0
Neuroendocrine malignant tumor	0	-	0	0	-	0	0	-	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0
Intestine	0	-	0	0	-	2	2.0	1	1.0	1	1.0	1	0.7	1	0.7	0	-	0	0	0	0	0	0	0	0	0	0	0
Adenomatous polyp	0	-	0	0	-	0	0	-	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0
Fibroma	0	-	0	0	-	0	0	-	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0
Leiomyoma	0	-	0	0	-	0	0	-	0	0	0	0	0	0	0	-	2	1.3	0	-	0	0	0	0	0	0	0	0
Adenocarcinoma	0	-	0	0	-	0	0	-	0	0	0	0	0	0	0	-	1	0.7	1	0.7	0	-	0	0	0	0	0	0
Leiomyosarcoma	0	-	0	0	-	1	1.0	0	-	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0

Continued.

TABLE 1. Continued.

Site	Groups													
	E: 100,000 ppm		II: 50,000 ppm		III: 10,000 ppm		IV: 2,000 ppm		V: 400 ppm		VI: 80 ppm		VII: 0 ppm (control)	
	No.	%	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Salivary glands														
Adenoma	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Basal cell carcinoma	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Liposarcoma & fibroliposarcoma	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Liver														
Hepatoma	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Cholangioma	0	-	4	4.0	1	1.0	2	2.0	2	2.0	4	4.0	2	2.0
Fibrosarcoma	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Hepatocarcinoma	1	1.0	0	-	0	-	0	-	0	-	0	-	0	-
Cholangiocarcinoma	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Hemangiosarcoma	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Pancreas														
Exocrine adenoma	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Islet cell adenoma	9	9.0	0	-	6	6.0	1	1.0	7	7.0	0	-	5	5.0
Islet cell carcinoma	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Kidneys														
Adenoma	0	-	1	1.0	0	-	0	-	0	-	0	-	0	-
Nephroma	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Fibrosarcoma	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Tubular cell carcinoma	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Pelvis ^c														
Papilloma	0	-	3	3.0	0	-	1	1.0	1	1.0	4	4.0	0	-
Transitional cell carcinoma	1	1.0	4	4.0	1	1.0	3	3.0	1	1.0	3	3.0	0	-
Bladder														
Papilloma	0	-	0	-	1	1.0	0	-	0	-	0	-	0	-
Transitional cell carcinoma	0	-	0	-	1	1.0	0	-	2	2.0	0	-	0	-
Prostate														
Adenoma	1	1.0	2	2.0	0	-	2	2.0	0	-	0	-	0	-
Seminal vesicles														
Rhabdomyosarcoma	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Testes														
Interstitial cell adenoma	1	1.0	3	3.0	0	-	2	2.0	0	-	6	6.0	3	3.0
Interstitial cell carcinoma	1	1.0	0	-	0	-	0	-	0	-	0	-	0	-

Continued

TABLE 1. Continued.

Site	Groups																															
	I: 100,000 ppm				II: 50,000 ppm				III: 10,000 ppm				IV: 2,000 ppm				V: 400 ppm				VI: 80 ppm				VII: 0 ppm (control)							
	Male	Female	No.	%	Male	Female	No.	%	Male	Female	No.	%	Male	Female	No.	%	Male	Female	No.	%	Male	Female	No.	%	Male	Female	No.	%				
Ovaries																																
Adenoma & cystadenoma	1	1.0	0	—	0	—	0	—	1	1.0	0	—	8	5.3	2	1.3	2	1.3	0	—	4	2.7	0	—	2	1.3	0	—	5	3.3	0	—
Granulosa &/or theca cell tumor	2	2.0	0	—	0	—	0	—	0	—	0	—	2	1.3	0	—	2	1.3	0	—	0	—	0	—	0	—	0	—	1	0.7	0	—
Granular cell tumor	0	—	0	—	0	—	0	—	1	1.0	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—
Seroli cell tumor	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—
Fibroma	1	1.0	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—
Fibrosarcoma	3(4)	3.0	3	3.0	1	1.0	0	—	1	1.0	0	—	1	0.7	0	—	1	0.7	0	—	0	—	0	—	0	—	0	—	0	—	0	—
Adenocarcinoma	1	1.0	1	1.0	0	—	0	—	0	—	0	—	1	0.7	0	—	1	0.7	0	—	1	0.7	0	—	0	—	0	—	0	—	0	—
Malignant Seroli cell tumor	0	—	0	—	0	—	0	—	0	—	0	—	1	0.7	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—
Uterus																																
Poly	42	42.0	39	39.0	0	—	0	—	28	28.0	23	15.3	35	23.3	35	23.3	51	34.0	51	34.0	51	34.0	51	34.0	38	25.3	38	25.3	2	1.3	2	1.3
Adenoma & fibroadenoma	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—
Fibroma	0	—	2	2.0	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—
Fibrosarcoma	1	1.0	1	1.0	1	1.0	1	1.0	2	1.3	0	—	0	—	0	—	1	0.7	0	—	1	0.7	0	—	1	0.7	0	—	3	2.0	3	2.0
Leiomyoma	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—
Squamous cell carcinoma	0	—	0	—	0	—	0	—	2	2.0	2	1.3	1	0.7	0	—	1	0.7	0	—	0	—	0	—	0	—	0	—	0	—	0	—
Adenocarcinoma	2	2.0	4	4.0	0	—	0	—	1	1.0	3	2.0	1	0.7	0	—	0	—	0	—	0	—	0	—	0	—	0	—	3	2.0	3	2.0
Hemangiosarcoma	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—
Leiomyosarcoma	0	—	1	1.0	0	—	0	—	0	—	0	—	1	0.7	0	—	1	0.7	0	—	0	—	0	—	0	—	0	—	1	0.7	0	—
Sarcoma botryoides	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—
Malignant schwannoma	3	3.0	1	1.0	1	1.0	4	4.0	4	4.0	3	2.0	2	1.3	4	2.7	2	1.3	2	1.3	2	1.3	2	1.3	6	4.0	6	4.0	0	—	0	—
Vagina																																
Poly	1	1.0	1	1.0	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	2	1.3	0	—	0	—	0	—
Acanthoma	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—
Fibroma	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—
Fibrosarcoma	0	—	0	—	0	—	0	—	0	—	0	—	1	0.7	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—
Benign schwannoma	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—
Hemangiosarcoma	0	—	0	—	0	—	0	—	0	—	0	—	1	0.7	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—
Peritonium																																
Lipoma & fibrolipoma	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—
Fibrosarcoma	0	—	1	1.0	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—
Mesothelioma	0	—	1	1.0	2	2.0	0	—	0	—	0	—	3	2.0	1	0.7	4	2.7	0	—	0	—	0	—	1	0.7	1	0.7	0	—	0	—
Hemangiosarcoma	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—
Pharyngeal gland																																
Adenoma	35	35.0	32	32.0	45	45.0	35	35.0	53	53.0	27	27.0	54	36.0	37	24.7	55	36.7	34	22.7	57	38.0	29	19.3	66	44.0	51	34.0	0	—	0	—
Adenocarcinoma	2	2.0	0	—	1	1.0	3	3.0	2	2.0	1	1.0	6	4.0	3	2.0	1	0.7	3	2.0	5	3.3	1	0.7	1	0.7	1	0.7	0	—	0	—
Thyroid gland																																
Follicular adenoma	0	—	1	1.0	0	—	1	1.0	0	—	1	0.7	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—
C-cell adenoma	5	5.0	5(6)	5.0	5	5.0	2	2.0	2	2.0	7	7.0	6	4.0	8(9)	5.3	6	4.0	7	4.7	4	2.7	5(6)	3.3	3	2.0	7(8)	4.7	0	—	0	—
Fibrosarcoma	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—
C-cell carcinoma	0	—	2	2.0	0	—	2	2.0	3	3.0	0	—	2	1.3	2(3)	1.3	2	1.3	3	2.0	0	—	2	1.3	1	0.7	2	1.3	0	—	0	—
Parathyroid gland																																
Adenoma	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	1	0.7	0	—	1	0.7	1	0.7	2	1.3	0	—	0	—	0	—

Continued.

TABLE 1. Continued.

Site	Groups																									
	I: 100,000 ppm			II: 50,000 ppm			III: 10,000 ppm			IV: 2,000 ppm			V: 400 ppm			VI: 80 ppm			VII: 0 ppm (control)							
	No.	%		No.	%		No.	%		No.	%		No.	%		No.	%		No.	%		No.	%			
Headtype																										
Headtype																										
Adenoma	2	2.0	8	8.0	2	2.0	2(4)	2.0	5	5.0	7	7.0	4	2.7	11(13)	7.3	1	0.7	9	6.0	2	1.3	10(11)	6.7	4	2.7
Cortical adenoma	19(22)	19.0	29(34)	29.0	21(25)	21.0	24(32)	24.0	19(26)	19.0	24(28)	24.0	15(18)	10.0	37(45)	24.7	25(28)	16.7	22(28)	14.7	20(22)	13.3	26(28)	17.3	18	12.0
Phaeochromocytoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gangliocytoma	0	0	1	1.0	0	0	2	2.0	0	0	2	2.0	0	0	2	1.3	0	0	2	1.3	0	0	2	1.3	1	0.7
Cortical adenocarcinoma	2	2.0	0	0	4	4.0	5	5.0	1	1.0	1(2)	1.0	0	0	2	1.3	0	0	2	1.3	0	0	4	2.7	0	0
Phaeochromoblastoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ganglioneuroblastoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Central nervous system																										
-Brain																										
Granular cell tumor	0	0	0	0	0	0	0	0	2	2.0	1	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Astrocytoma	0	0	1	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oligodendroglioma	1	1.0	0	0	2	2.0	0	0	0	0	0	0	0	1	0.7	0	0	0	0	0	0	0	2	1.3	1	0.7
Multiform glioblastoma	0	0	0	0	0	0	1	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Meningeal medulloblastoma	0	0	0	0	0	0	0	0	0	0	1	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Meninges																										
Benign meningioma	1	1.0	1	1.0	5	5.0	1	1.0	2	2.0	1	1.0	2	1.3	2	1.3	3	2.0	2	1.3	1	0.7	1	0.7	2	1.3
Malignant meningioma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Peripheral nervous system																										
-Cranial nerves																										
Benign gangliocytoma	0	0	0	0	1	1.0	0	0	0	0	0	0	0	0	0	0	1	0.7	0	0	0	0	0	0	0	0
Benign schwannoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1.3	0	0	0	0	0	0	0	0	0	0
Malignant schwannoma	3	3.0	1	1.0	3	3.0	1	1.0	2	2.0	1	1.0	2	1.3	1	0.7	1	0.7	1	0.7	0	0	1	0.7	1	0.7
-Other peripheral nerves																										
Malignant schwannoma	1	1.0	1	1.0	0	0	0	0	0	0	0	0	0	2	1.3	2	1.3	0	0	0	0	0	1	0.7	0	0
Bones																										
-Head																										
Chondroma	0	0	0	0	0	0	0	0	1	1.0	1	1.0	1	0.7	0	0	0	0	0	0	0	0	0	0	0	0
Osteoma	0	0	2	2.0	0	0	0	0	3	3.0	1	1.0	1	0.7	1	0.7	2	1.3	1	0.7	4	2.7	1	0.7	0	0
Osteosarcoma	3	3.0	4	4.0	2	2.0	3	3.0	5	5.0	3	3.0	9	6.0	4	2.7	5	3.3	3	2.0	7	4.7	9	6.0	5	3.3

Continued

statistical analysis, were described in detail in previous publications.^{23,24} The experiment was conducted according to the Italian law regulating the use of animals for scientific purposes.²⁵

RESULTS

The study proceeded smoothly without unexpected occurrences. The bi-phase ended at 151 weeks, with the death of the last animal at the age of 159 weeks. Results of the study are reported in previous publications.^{23,24}

Water consumption did not differ among males and females of treated and control groups. A dose-related difference in food consumption was observed in both sexes during the experiment. A slight decrease in body weight was observed in females treated at the highest dose; no substantial differences were observed among treated males, compared to controls. No differences were observed in survival among males or females of the treated groups, compared to controls.

The occurrence of benign and malignant tumors among male and female rats is shown in Table 1. The differences observed among treated and control animals were as follows:

1. an increase in malignant tumor-bearing animals with a significant positive trend in males ($P \leq 0.05$) and in females ($P \leq 0.01$) and a statistically significant difference in females treated at 50,000 ppm ($P \leq 0.01$), compared to controls (Table 2);
2. an increased incidence of hyperplasia of the olfactory epithelium with a significant positive trend in males and females (Table 3). It is noteworthy that among females treated at the highest dose, one case of dysplastic hyperplasia, one adenoma, and one olfactory neuroblastoma were observed. The neuroblastoma invaded the cranium, compressing the forebrain and was positive for chromogranin A immunohistochemical staining;
3. an increase in the incidence of dysplastic hyperplasias, dysplastic papillomas, and carcinomas of the renal pelvis and ureter were observed in females (Table 4). Carcinomas in females occurred with a positive trend ($P \leq 0.05$) and specifically in females exposed at 100,000 ppm ($P \leq 0.05$), compared with controls. Carcinomas were also observed among males treated at 100,000, 50,000, 10,000, and 2000 ppm. In females, when dysplastic lesions and carcinomas are combined, they show a significant positive trend ($P \leq 0.01$) and a statistically significant increase in those treated at 100,000 ($P \leq 0.01$), 50,000 ($P \leq 0.01$), 10,000 ($P \leq 0.01$), 2000 ($P \leq 0.05$), and 400 ppm ($P \leq 0.05$). An increased incidence of deposits of calcium (mineralization) was observed in females, particularly in those treated at 100,000 ppm (39%), 50,000 ppm (25%), or 10,000 ppm

TABLE 2. Long-term carcinogenicity bioassay on ASPARTAME, administered with feed, supplied *ad libitum*, to male (M) and female (F) Sprague-Dawley rats

MALIGNANT TUMORS					
Group No.	Concentration (ppm)	Animals		Tumor-bearing animals ^{a,b,c,d}	
		Sex	No.	No.	%
I	100,000	M	100	43	43.0
		F	100	51	51.0
		M+F	200	94	47.0
II	50,000	M	100	38	38.0
		F	100	58	58.0 ^{##}
		M+F	200	96	48.0
III	10,000	M	100	34	34.0
		F	100	40	40.0
		M+F	200	74	37.0
IV	2,000	M	150	60	40.0
		F	150	67	44.7
		M+F	300	127	42.3
V	400	M	150	48	32.0
		F	150	70	46.7
		M+F	300	118	39.3
VI	80	M	150	44	29.3
		F	150	64	42.7
		M+F	300	108	36.0
VII	0 (control)	M	150	53	35.3*
		F	150	55	36.7**
		M+F	300	108	36.0

^aThe tumors rates are based on the number of animals examined (necropsied).

^b*P-Values* corresponding to pairwise comparisons 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.

^dBilateral and multiple tumors were plotted as single independent tumors.

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

**Statistically significant ($P < 0.01$) using Cochran-Armitage trend test.

##Statistically significant ($P < 0.01$) using Poly-*k* test ($k = 3$).

(19%), compared with controls (8%). The same effect was not observed among males of the various groups. No difference was observed in the incidence of acute and chronic nephropathies among males and females of all groups. It must be noted that the nephropathy is common in the natural dying process and for this reason, is more frequently observed when animals are allowed to die spontaneously;

4. a dose-related increased incidence in malignant schwannomas of peripheral nerves was observed, with a significant positive trend in males ($P \leq 0.05$), while in females, nine malignancies were observed among treated animals of the different dosage groups and none among controls (Table 5). All lesions, in males and females, diagnosed as malignant schwannoma, were positive for S100 immunohistochemical staining. The occurrence

TABLE 3. Long-term carcinogenicity bioassay on ASPARTAME, administered with feed, supplied *ad libitum*, to male (M) and female (F) Sprague-Dawley rats

		PRENEOPLASTIC AND NEOPLASTIC LESIONS OF OLFACTORY EPITHELIUM							
Group No.	Concentration (ppm)	Animals		Animals with preneoplastic and neoplastic lesions ^{a,b,c}				Olfactory neuroblastoma	
		Sex	No.	Hyperplasia		Adenoma		No.	%
				No.	%	No.	%	No.	%
I	100,000	M	100	14	14.0 ^{##}	0	—	0	—
		F	100	19 ^d	19.0 ^{##}	1	1.0	1	1.0
		M+F	200	33	16.5	1	0.5	1	0.5
II	50,000	M	100	12	12.0 ^{##}	0	—	0	—
		F	100	21	21.0 ^{##}	0	—	0	—
		M+F	200	33	16.5	0	—	0	—
III	10,000	M	100	7	7.0 ^{##}	2	2.0	0	—
		F	100	17	17.0 ^{##}	0	—	0	—
		M+F	200	24	12.0	2	1.0	0	—
IV	2,000	M	150	4	2.7	1	0.7	0	—
		F	150	13	8.7	1	0.7	0	—
		M+F	300	17	5.7	2	0.7	0	—
V	400	M	150	9	6.0 ^{##}	0	—	0	—
		F	150	11	7.3	1	0.7	0	—
		M+F	300	20	6.7	1	0.3	0	—
VI	80	M	150	3	2.0	0	—	0	—
		F	150	5	3.3	2	1.3	0	—
		M+F	300	8	2.7	2	0.7	0	—
VII	0 (control)	M	150	1	0.7 ^{###}	0	—	0	—
		F	150	6	4.0 ^{###}	0	—	0	—
		M+F	300	7	2.3	0	—	0	—

^aThe tumors rates are based on the number of animals examined (necropsied).

^b*P*-Values corresponding to pairwise comparisons 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.

^dOne hyperplasia with atypia.

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

##Statistically significant ($P < 0.01$) using Poly-*k* test ($k = 3$).

of malignant schwannomas mostly involved cranial nerves (72%). The other cases arose from spinal nerve roots. Among three males treated at the highest dose, metastases were observed in the submandibular lymph nodes in two cases, and in the lung and liver in the third case;

5. a dose-related increased incidence in lymphomas-leukemias was observed, with a significant positive trend in males ($P \leq 0.05$) and in females ($P \leq 0.01$). When compared to controls, a statistically significant difference was observed in females treated at doses of 100,000

TABLE 4. Long term carcinogenicity bioassay on ASPARTAME, administered with feed, supplied *ad libitum*, to male (M) and female (F) Sprague-Dawley rats

		PRENEOPLASTIC AND NEOPLASTIC LESIONS OF THE TRANSITIONAL CELL EPITHELIUM OF THE RENAL PELVIS AND URETER									
Group No.	Concentration (ppm)	Animals		Animals with preneoplastic or neoplastic lesions ^{a,b,c,d}						Total	
		Sex	No.	Dysplastic hyperplasias		Dysplastic papillomas		Carcinomas		No.	%
				No.	%	No.	%	No.	%		
I	100,000	M	100	3	3.0	0	—	1	1.0	4	4.0
		F	100	8	8.0	3	3.0	4	4.0[#]	15	15.0^{##}
		M+F	200	11	5.5	3	1.5	5	2.5	19	9.5
II	50,000	M	100	2	2.0	0	—	1	1.0	3	3.0
		F	100	6	6.1	1	1.0	3	3.0	10	10.1^{##}
		M+F	200	8	4.0	1	0.5	4	2.0	13	6.5
III	10,000	M	100	2	2.0	0	—	1	1.0	3	3.0
		F	100	6	6.0	1	1.0	3 ^d	3.0	10	10.0^{##}
		M+F	200	8	4.0	1	0.5	4	2.0	13	6.5
IV	2,000	M	150	4	2.7	0	—	1	0.7	5	3.3
		F	150	6	4.0	1	0.7	3 ^d	2.0	10	6.7[#]
		M+F	300	10	3.3	1	0.3	4	1.3	15	5.0
V	400	M	150	4	2.7	1	0.7	0	—	5	3.4
		F	150	5	3.3	1	0.7	3	2.0	9	6.0[#]
		M+F	300	9	3.0	2	0.7	3	1.0	14	4.7
VI	80	M	150	3	2.0	0	—	0	—	3	2.0
		F	150	4	2.7	1	0.7	1	0.7	6	4.0
		M+F	300	7	2.3	1	0.3	1	0.3	9	3.0
VII	0 (control)	M	150	1	0.7	0	—	0	—	1	0.7
		F	150	2	1.3	0	—	0	— [#]	2	1.3^{**##}
		M+F	300	3	1.0	0	—	0	—	3	1.0

^aThe tumor rates are based on the number of animals examined (necropsied).
^b*p*-Values corresponding to pairwise comparisons 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.
^dOne animal bears bilateral tumor.
^{*}Statistically significant (*P* < 0.05) using Cochran-Armitage trend test.
^{**}Statistically significant (*P* < 0.01) using Cochran-Armitage trend test.
[#]Statistically significant (*P* < 0.05) using Poly-k test (*k* = 3).
^{##}Statistically significant (*P* < 0.01) using Poly-k test (*k* = 3).

(*P* ≤ 0.01), 50,000 (*P* ≤ 0.01), 10,000 (*P* ≤ 0.05), 2000 (*P* ≤ 0.05), and 400 (*P* ≤ 0.01) ppm (Table 6). Lymphomas–leukemias are neoplasias arising from hemolymphoreticular tissues and their aggregation is widely used in experimental carcinogenesis. The reason is that both solid and circulating phases are present in many lymphoid neoplasms, and the distinction between them is artificial.²⁶

TABLE 5. Long-term carcinogenicity bioassay on ASPARTAME, administered with feed, supplied *ad libitum*, to male (M) and female (F) Sprague-Dawley rats

MALIGNANT SCHWANNOMAS OF PERIPHERAL NERVES									
Group No.	Concentration (ppm)	Animals		Animals with tumors ^{a,b,c}					
		Sex	No.	Cranial nerves		Other nerves		Total	
				No.	%	No.	%	No.	%
I	100,000	M	100	3	3.0	1	1.0	4	4.0
		F	100	1	1.0	1	1.0	2	2.0
		M+F	200	4	2.0	2	1.0	6	3.0
II	50,000	M	100	3	3.0	0	—	3	3.0
		F	100	1	1.0	0	—	1	1.0
		M+F	200	4	2.0	0	—	4	2.0
III	10,000	M	100	2	2.0	0	—	2	2.0
		F	100	1	1.0	0	—	1	1.0
		M+F	200	3	1.5	0	—	3	1.5
IV	2,000	M	150	2	1.3	0	—	2	1.3
		F	150	1	0.7	2	1.3	3	2.0
		M+F	300	3	1.0	2	0.7	5	1.7
V	400	M	150	1	0.7	2	1.3	3	2.0
		F	150	0	—	0	—	0	—
		M+F	300	1	0.3	2	0.7	3	1.0
VI	80	M	150	1	0.7	0	—	1	0.7
		F	150	1	0.7	1	0.7	2	1.3
		M+F	300	2	0.7	1	0.3	3	1.0
VII	0 (control)	M	150	1	0.7	0	—	1	0.7*#
		F	150	0	—	0	—	0	—
		M+F	300	1	0.3	0	—	1	0.3

^aThe tumors rates are based on the number of animals examined (necropsied).

^b*P*-Values corresponding to pairwise comparisons 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 ($P < 0.05$) using Cochran-Armitage trend test.

#Statistically significant ($P < 0.05$) using Poly-*k* test ($k = 3$).

Concerning the incidence of brain malignant tumors, a controversial issue in the experiments performed in the 1970s and early 1980s, 12 malignant tumors (10 gliomas, 1 medulloblastoma, and 1 meningioma) were observed in our study, without dose relationship, in males and females treated with APM, while none were observed in controls.

CONCLUSIONS

In our experimental conditions, APM causes an increased incidence of malignant tumor-bearing animals, with a positive significant trend in both sexes and a significant increase in the incidence of tumors at various sites, including carcinomas of the renal pelvis and ureter in females, malignant schwannomas

TABLE 6. Long-term carcinogenicity bioassay on ASPARTAME, administered with feed, supplied *ad libitum*, to male (M) and female (F) Sprague-Dawley rats

HEMOLYMPHORETICULAR NEOPLASIAS					
Group No.	Concentration (ppm)	Animals		Animals with lymphomas–leukemias ^{a,b,c}	
		Sex	No.	No.	%
I	100,000	M	100	29	29.0
		F	100	25	25.0 ^{##}
		M+F	200	54	27.0
II	50,000	M	100	20	20.0
		F	100	25	25.0 ^{##}
		M+F	200	45	22.5
III	10,000	M	100	15	15.0
		F	100	19	19.0 [#]
		M+F	200	34	17.0
IV	2,000	M	150	33	22.0
		F	150	28	18.7 [#]
		M+F	300	61	20.3
V	400	M	150	25	16.7
		F	150	30	20.0 ^{##}
		M+F	300	55	18.3
VI	80	M	150	23	15.3
		F	150	22	14.7
		M+F	300	45	15.0
VII	0 (control)	M	150	31	20.7 ^{*#}
		F	150	13	8.7 ^{*##}
		M+F	300	44	14.7

^aThe tumors rates are based on the number of animals examined (necropsied).

^b*P*-Values corresponding to pairwise comparisons 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 ($P < 0.05$) using Cochran-Armitage trend test.

**Statistically significant ($P < 0.01$) using Cochran-Armitage trend test.

#Statistically significant ($P < 0.05$) using Poly-*k* test ($k = 3$).

##Statistically significant ($P < 0.01$) using Poly-*k* test ($k = 3$).

of the peripheral nerves in males, and lymphomas–leukemias in females. The carcinogenic effects were shown even at a daily dose of 20 mg/kg b.w., about half the current ADI for humans in Europe and the United States.

The results of our study are not consistent with the data made available by the producers of APM. The interpretation of our results and the explanation for this difference have been extensively discussed in our previous publications.^{23,24} The distinctive characteristics of the CMCRC/ERF long-term carcinogenicity bioassays, that is, that they are planned using a large number of animals per sex and per group and that animals are observed until spontaneous death have

been, in our opinion, critical. Had we truncated the experiment after just 2 years, we would have most likely not revealed the carcinogenic evidence of APM.

The results of our study demonstrate the necessity of an extensive review of the regulations governing the use of APM as a food additive. The data also call for additional long-term bioassays on another species and a different calendar of exposure to better quantify APM's carcinogenic risk. In our opinion, it is of vital importance to also re-analyze the adequacy of the long-term carcinogenicity bioassays performed on other old- and new-generation artificial sweeteners currently in use worldwide.

Given the ever-increasing use of artificial sweeteners in both industrialized and developing countries, we consider our integrated project on artificial sweeteners to be of the highest priority for the protection of public health, in particular the health of children and pregnant women who are among the most vulnerable populations. In light of this goal, and given what in our view are inadequate data to date on the carcinogenicity of artificial sweeteners, we are conducting additional research, not only on APM, but also on other widely diffused artificial sweeteners and blends used in thousands of foods, beverages, and pharmaceutical products.

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