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SCIENTIFIC OPINION

Opinion on a request from the European Commission related to the 2nd ERF carcinogenicity study on aspartame [1]

Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food

(EFSA-Q-2008-746)

Adopted on 29 January 2009

This opinion replaces the earlier version published on 20 April 2009.[2]

PANEL MEMBERS*

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SUMMARY

Following a request from the European Commission, the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion on the results of a long-term carcinogenicity study with prenatal exposure to the artificial sweetener aspartame, performed by The Cesare Maltoni Cancer Research Center of the European Ramazzini Foundation (ERF) and published in June 2007 by Soffritti *et al.* The authors concluded that the results of their study not only confirm, but also reinforce their first experimental demonstration (published in 2005 and 2006) of aspartame's multipotential carcinogenicity at a dose level close to the human Acceptable Daily Intake (ADI). Based on the results of this study, the authors further postulated that when lifespan exposure to aspartame begins during fetal life, its carcinogenic effects are increased.

During the 1980s, aspartame has been authorised for use in foods and as a table-top sweetener by several Member States, and European legislation harmonising its use in foodstuffs was

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[2] Editorial changes only: page 9 the figure referring to total number of tumors in the 2000 ppm aspartame group in table 1 has been changed from 1 to 31 . The changes do not affect the overall conclusion of the opinion. To avoid confusion, the original version has been removed from the website.

* one member of the Panel did not participate in the discussion on the subject referred to above because of possible conflicts of interest.

introduced in 1994 following thorough safety evaluations by the Scientific Committee on Food (SCF) in 1984 and 1988. Further reviews of aspartame data were carried out by the SCF in 1997 and 2002. In 2006, the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) assessed a long-term carcinogenicity study on aspartame performed by the ERF and published by Soffritti *et al.* in 2005 and 2006. Based on all the evidence available from the ERF study and other recent studies and previous evaluations, the AFC Panel concluded that there was no reason to revise the previously established ADI for aspartame of 40 mg/kg bw (EFSA, 2006).

In the second ERF study on aspartame in rats, published in 2007, dietary concentrations of 400 and 2000 mg aspartame/kg diet equivalent to doses of 20 and 100 mg aspartame/kg bw/day were used. The rats were exposed to aspartame from the 12th day of gestation until natural death. The group size was 95/sex in the control and 70/sex in the low- and high-dose groups. The authors reported a significant dose-related increase of malignant tumour-bearing males, particularly in the high-dose group ($p<0.01$, Cox regression model), a significant increase in incidence of lymphomas/leukaemias in males from the high-dose group ($p<0.05$), a significant dose-related increase in incidence of lymphomas/leukaemias in females ($p<0.01$), particularly in the high-dose group ($p<0.01$), and a significant dose-related increase in incidence of mammary carcinomas in females ($p<0.05$), particularly in the high-dose group ($p<0.05$).

The Panel's assessment of the ERF carcinogenicity study with prenatal exposure on aspartame as reported by Soffritti *et al.* was directed towards establishing the relevance of the reported findings to human health. In carrying out its assessment the Panel only had access to the published paper, in which the presentation of pathological findings was restricted to the incidence of malignant tumours, total number of malignant tumours per group, incidence of lymphomas/leukaemias, and incidence of mammary carcinomas. Neither further data from this study nor an explanation on the analytical method used, were provided by the authors to EFSA by the time of the adoption of this opinion.

The Panel concluded that:

- Evaluation of aggregated malignant tumour incidences as evidence of carcinogenic potential of the test compound can only be performed based on a thorough consideration of all tumour data including onset, and data on non-neoplastic, hyperplastic and preneoplastic lesions but these data were not provided by the authors.
- In accordance with the previous view of the AFC Panel, the lymphomas and leukaemias might have developed in a population of rats suffering from chronic respiratory disease.
- The increase in incidence of mammary carcinoma is not considered indicative of a carcinogenic potential of aspartame since the incidence of mammary tumours in female rats is rather high and varies considerably between carcinogenicity studies. The Panel also noted that an increased incidence of mammary carcinomas was not reported in the previous ERF study with aspartame which used much higher doses of the compound.

Overall, the Panel concluded, on the basis of all the evidence currently available from this ERF study and previous evaluations, that there is no indication of any genotoxic or carcinogenic potential of aspartame and that there is no reason to revise the previously established ADI for aspartame of 40 mg/kg bw.

Key words:

Aspartame, L-aspartyl-L-phenylalanine methyl ester, artificial sweetener, life-long study, prenatal exposure, CAS No. 22839-47-0, E 951, intense sweetener

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

In June 2005**,** the European Food Safety Authority (EFSA) was informed about the outcome of a long-term carcinogenicity study on the sweetener aspartame, carried out by The Cesare Maltoni Cancer Research Center, European Ramazzini Foundation of Oncology and Environmental Sciences, Bologna, Italy (the Ramazzini Foundation, ERF). The ERF considered that the results of their study indicate that aspartame is a multipotential carcinogenic agent, and recommended that a re-evaluation of the present guidelines on the use and consumption of aspartame should be undertaken. EFSA, following a request from the European Commission on 1 July 2005, requested its Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) to review these findings, as a matter of high priority.

In June 2007 a paper entitled "Lifespan exposure to low doses of aspartame beginning during prenatal life increases cancer effect in rats" by Soffritti *et al.*, was published online in Environmental Health Perspectives. The paper was based on results of a second carcinogenicity study on aspartame carried out by the ERF. EFSA has informed the Health and Consumers Directorate General about this second study on aspartame that concludes that when lifespan exposure to aspartame begins during foetal life, its carcinogenic effects are increased.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1) (a) and Article 31 of Regulation (EC) No 178/2002, the European Commission requested the European Food Safety Authority to extend the previous terms of reference submitted to EFSA on 1 July 2005:

- to assess the new study published in 2007 and
- depending on the outcome of this assessment, to review the previous opinion on the safety of aspartame, in the light of the new study.

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ASSESSMENT

1. Introduction

The sweetener aspartame has been authorised for use in foods and as a table-top sweetener by several Member States, and European legislation harmonising its use in foodstuffs was introduced in 1994 following thorough safety evaluations by the Scientific Committee on Food in 1984 and 1988 (SCF, 1985; 1989). The safety of aspartame has been extensively investigated through clinical and laboratory research, intake studies and post-marketing surveillance. However, since its approval the safety of aspartame has been repeatedly questioned. Further reviews of data on aspartame were carried out by the SCF in 1997 and 2002 (SCF, 1997; 2002). In 2006, at the request of the European Commission, the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC), assessed a long-term carcinogenicity study on aspartame performed by the the European Ramazzini Foundation (ERF) (Soffritti *et al.*, 2005; 2006), with particular emphasis on the relevance of the reported findings for human health. For that evaluation the AFC Panel received a full report on the study (Soffritti and Belpoggi, 2005). On the basis of all the evidence available from the ERF study, other recent studies and previous evaluations, the overall conclusion of the AFC Panel was that there was no reason to revise the previously established Acceptable Daily Intake (ADI) for aspartame of 40 mg/kg bw (EFSA, 2006).

In assessing the present ERF carcinogenicity study on aspartame, the Scientific Panel on Food Additives and Nutrient Source added to Food (ANS) only had access to the published paper by Soffritti *et al.* (2007). In this paper the presentation of pathological findings was restricted to the incidences of total malignant tumours, lymphomas/leukaemias, and mammary carcinomas. Further data from this study were requested by EFSA in April 2007, January 2008 and July 2008 in order to aid the interpretation of the study, but were not provided by the ERF by the time of adoption of this opinion.

2. Study design and conduct

The study design and conduct was described in the Soffritti *et al.* (2007) publication.

The aspartame used was produced by Ajinomoto. Its purity was >98.7%, with a specification for diketopiperazine of <0.3% and L-phenylalanine of <0.5%. The stability of the aspartame in feed was analysed prior to the start of the study and periodically confirmed throughout the course of the study.

Sprague Dawley rats from the in-bred colony of the ERF were organised into three groups: an untreated control group (95 males and 95 females), a low-dose group (70 males and 70 females) receiving aspartame at a dietary concentration of 400 mg/kg, equivalent to a daily dose of 20 mg/kg bw, and a high-dose group (70 males and 70 females) receiving aspartame at a dietary concentration of 2000 mg/kg, equivalent to a daily dose of 100 mg/kg bw. The exposure to aspartame began prenatally on the 12th day of gestation (when organogenesis is completed and before which time many tissues and organs are refractory to the effects of carcinogenic agents). After weaning, five males or five females were housed per cage (corresponding to an area of approximately 200 cm^2), at a temperature of 23 ± 2 ºC and relative humidity of 50-60%. No information on the light cycle was provided in the Soffritti *et al.* (2007) publication. The feed was supplied *ad libitum*. The study continued until the death

of all the test animals, which took place at the age of 144 weeks (=end of the biophase of the 147 weeks study).

The following parameters were recorded:

- water and feed consumption (mean per cage),
- body weight (for rats aged 6 weeks old, once weekly for the first 13 weeks of the study and then once every two weeks until the end of the experiment),
- mortality (survival),
- clinical observations (status, behaviour, natural death; 3 times daily on working days, twice daily during weekends and holidays),
- clinical examination for gross lesions (every 2nd week),
- general pathological lesions, macroscopic (at necropsy) and microscopic,
- types of tumours and tumour precursors,
- number and percentage of animals bearing malignant tumours,
- number of malignant tumours per group and per 100 animals,
- cumulative prevalence by age of death of female rats bearing hemolymphoreticular neoplasias.

All animals were subjected to complete necropsy. 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. Histopathology was routinely performed on a comprehensive range of organs and tissues from each animal from each group. Soffritti *et al.* (2007) report that all slides were examined and evaluated histopathologically by the same group of pathologists, following the same criteria for histopathological evaluation and classification, after which a senior pathologist reviewed all tumours and all other lesions of oncological interest.

Soffritti *et al.* (2007) briefly describe the statistical method used: *"We performed statistical evaluations of the incidence and dose–response relationship of neoplastic lesions using the Cox regression model* (Cox 1972)*. p-Values are reported in the tables."*

3. The ANS Panel's comments on study design and conduct

The Panel noted that there is no information on whether the study was performed under Good Laboratory Practice (GLP) conditions.

The study design does not refer to a specific Test Guideline being followed for performing a long-term carcinogenicity study. For the purpose of the present evaluation, the conduct of the study has been compared with the OECD Test Guideline 451 "Carcinogenicity Studies" (OECD, 1981).

The design of the ERF carcinogenicity bioassay included aspartame exposure from the second stage of gestation up to the natural death of all the animals, i.e. for the lifespan of the animals. This contrasts with the recommendation in the OECD Test Guideline 451 for the duration of the study, namely *"a study duration which covers the majority of the expected lifespan"* (2 years in the case of the rat). The number of animals per group was increased in the ERF study

compared to the minimum number recommended in the OECD Test Guideline 451, which is at least 50/sex/group.

The longer duration of the ERF study, covering the entire life span of the animals and the use of more animals per group may increase the sensitivity of the bioassay. However, life-long treatment also causes an increase in background pathology, which may confound the interpretation of the results. This especially relates to pathological findings such as leukemias/lymphomas and mammary gland tumours, the spontaneous incidences of which are high in laboratory animals from inbred colonies and are known to vary considerably between studies (Greaves, 2000).

In order to evaluate the results on malignant tumours, Soffritti *et al.* (2007) presented the number of tumour-bearing animals per group and percentages of tumour-bearing animals relative to the number of animals at the start of the study instead of the number of animals examined, which is commonly used. The use of the number of animals at the start of the experiment instead of the number of animals examined is only justified if no losses of organs/tissues occurred during the study.

Soffritti *et al.* (2007) used a Cox regression model for the statistical evaluation of the incidence and dose-response relationships of the neoplastic lesions. However, the authors did not explain in the publication the statistical approach used and have not provided EFSA with additional information. Therefore, the Panel was not in the position to assess the appropriateness of the statistical evaluation used.

4. Results as reported by the authors of the study

During the in-life phase of the study no relevant differences were observed in feed consumption for treated animals compared to the controls (Fig. 1A and B in Soffritti *et al.*, 2007). No differences were observed in water intake, or in mean body weight in the treated groups compared to the controls (Figure 1C in Soffritti *et al.*, 2007). Soffritti *et al.* (2007) reported "*a slight decrease, seemingly dose-related, in survival in the treated groups compared with the control group in both males and females*".

Presentation of the oncological results for males and females (Tables 1 and 2) were restricted to the incidence of animals bearing malignant tumours (number of malignant tumour-bearing animals and percentage per group), total number of malignant tumours per group (absolute value and calculated per 100 animals), incidence of animals bearing lymphomas/leukaemias (number of animals and percentage per group), and incidence of animals bearing mammary carcinomas (number of animals and percentage per group).

In relation to total malignant tumours, the authors reported that *"The incidence of malignant tumour bearing animals occurred with a significant, dose-related increase in males ($p \leq 0.01$). A significant increase of the incidence of malignant tumours was observed in males treated with 2000 ppm ($p \leq 0.01$) compared to the control group. Albeit not significant, a numeric increase in the incidence of animals bearing malignant tumours was also observed among females exposed to 2000 ppm compared to the controls."* Furthermore, the authors stated that the tumour types which contributed most to this increased incidence were lymphomas/leukemias and mammary carcinomas.

Table 1. Incidence of malignant tumors in male Sprague-Dawley rats exposed to APM from fetal day 12 throughout the life span (from Soffritti *et al.*, 2007).

APM dose, ppm (mg/kg bw)	No. of animals at start	Malignant tumors[a]				Total animals bearing lymphomas/leukemias[c]		Total animals bearing mammary carcinomas	
		Tumor-bearing animals[b]		Total tumors					
		No.	Percent	No.	No./100 animals	No.	Percent	No.	Percent
2,000 (100)	70	28	40.0**	31	44.3	12	17.1*	2	2.9
400 (20)	70	18	25.7	19	27.1	11	15.7	0	—
0 (0)	95	23	24.2**	26	27.4	9	9.5	0	—

[a] Tumor rates are based on the number of animals examined (necropsied). [b] p-Value associated with the dose–response test is near the control incidence. [c] In male historical controls (2,265 rats), the overall incidence of lymphomas/leukemias is 20.6% (range, 8.0–30.9%). *Significant ($p \leq 0.05$) using Cox regression model. **Significant ($p \leq 0.01$) using Cox regression model.
NB. APM: aspartame

Table 2. Incidence of malignant tumors in female Sprague-Dawley rats exposed to APM from fetal day 12 throughout the life span (from Soffritti *et al.*, 2007).

APM dose, ppm (mg/kg bw)	No. of animals at start	Malignant tumors [a]				Total animals bearing lymphomas/leukemias[b,c]		Total animals bearing mammary carcinomas[c]	
		Tumor-bearing animals[b]		Total tumors					
		No.	Percent	No.	No./100 animals	No.	Percent	No.	Percent
2,000 (100)	70	37	52.9	60	85.7	22	31.4**	11 (15)[d]	15.7*
400 (20)	70	31	44.3	44	62.9	12	17.1	5 (6)	7.1
0 (0)	95	42	44.2	48	50.5	12	12.6**	5 (6)	5.3*

[a]Tumor rates are based on the number of animals examined (necropsied). [b]In female historical controls (2,274 rats), the overall incidence of lymphomas/leukemias is 13.3% (range, 4.0–25.0%), and of mammary cancers is 9.2% (range, 4.0-14.2%). [c]p-Values associated with the dose–response test are near the control incidence. [d]Number of animals (number of tumors); an animal can bear multiple tumors. *Significant ($p \leq 0.05$) using Cox regression model. **Significant ($p \leq 0.01$) using Cox regression model.
NB. APM: aspartame

In relation to lymphomas/leukaemias, Soffritti *et al.* (2007) reported that "*the data show that aspartame causes a significant, dose-related increased incidence in females ($p \leq 0.01$). When compared with the untreated control group, the increased incidence of lymphomas/leukemias in treated males and females was significant at 2000 ppm aspartame ($p \leq 0.05$ and $p \leq 0.01$ respectively)*".

In relation to histotypes of lymphomas/leukemias, Soffritti *et al.* (2007) indicated that *"In males the most frequent histotypes observed were lymphoimmunoblastic lymphomas, that mainly involved lung and mediastinal/peripheral nodes. In females, the most frequent histotypes were lymphocitic lymphomas and lymphoimmunoblastic lymphomas that mainly involved the thymus, lung, spleen, and peripheral nodes."*

With regard to the differential diagnoses of lymphomas/leukemias, Soffritti *et al.* (2007) indicated that these *"were based on the morphological criteria regularly used in our laboratories, according to the guidelines of the international Classification of Rodent Tumors (IARC 1993). Lymphomas/leukaemias (this term includes all types of hemolymphosarcomas and leukemias) are neoplasia arising from hemolymphoreticular tissues. Their aggregation is*

regularly used in experimental carcinogenesis because both solid and circulating phases are present in many lymphoid neoplasms, and distinction between them is artificial (Harris et al. 2001)".

In relation to mammary carcinomas, Soffritti *et al.* (2007) reported a dose-related increase in the incidence of carcinomas in females ($p \leq 0.05$) and a significantly higher ($p \leq 0.05$) incidence of carcinomas in females exposed to the high-dose (100 mg/kg bw/day).

5. The ANS Panel's comments on the observed effects

5.1. General comments

Histopathological observations reported in the Soffritti *et al.* (2007) publication were restricted to the incidence and total numbers of malignant tumours, incidence of lymphomas/leukaemias, and incidence of mammary gland carcinomas. The Panel noted that no information was provided on the incidence of other types of malignant tumours, nor on the types of tumours included in the histopathological classification of the lymphomas and leukaemias, while the significant difference observed for lymphomas and leukaemias was considered by the authors to be one of the major findings of the study.

Incidence of non-neoplastic, pre-neoplastic and other neoplastic lesions were not presented. An overview of certain non-neoplastic lesions such as chronic inflammatory changes is essential for the interpretation of other pathological findings e.g. hyperplastic changes or neoplasms. Similarly, an overview of pre-neoplastic changes or benign neoplasms is required for the interpretation of the carcinogenic potential of the test compound. This information is particularly important for interpreting the results of life-long studies lasting up to the natural death of the animals, where increased background pathologies, such as infectious pathologies, or increased incidences of certain tumour types, e.g. pituitary tumours or lymphomas/leukaemias, are known to occur with increasing age.

5.2. Total malignant tumours

The Panel noted that aggregation of all malignant tumour incidences or aggregation of the total number of malignant tumours for statistical purposes, as performed by the authors of the ERF study, is not a scientifically sound approach.

The significant dose-related increase in the incidence of malignant tumour-bearing animals and the statistically significant increased incidence of malignant tumours in the high-dose group, both reported as percentages, were limited to males. The Panel noted that the percentages of malignant tumour-bearing males in the groups receiving 100 mg aspartame/kg bw in the present and the first ERF studies were comparable (40% in the present study versus 46% in the previous study), but the percentage of tumour-bearing control males in the present study (24.2%) was lower than in the first ERF study (39.3%). No data on the historical control values for malignant tumour-bearing animals in Sprague Dawley rats were given in the publication.

In the present ERF study, in all females treated with aspartame the incidence of malignant tumour-bearing animals was not statistically different to that in the control group, both when

data were expressed as total number of malignant tumour-bearing animals (absolute numbers) or as percentages.

The Panel noted that the total number of malignant tumours as recorded or calculated per 100 animals in the aspartame treated groups of both sexes was not statistically increased compared to the controls.

5.3. Lymphomas/leukaemias

The Panel noted that the incidence of lymphomas/leukaemias as reported by Soffritti *et al.* (2007) were statistically significantly increased only in the high-dose groups (100 mg/kg bw/day) of both sexes.

The Panel noted that the variation in the incidence of lymphomas/leukaemias in this strain of rats appears to be high. The lymphoma/leukaemia incidence in the female high-dose group (31.4%) was above the upper value for historical controls in the ERF laboratory (range: 4.0-25.0%, data collected over the last 20 years) but the increase was slight, as indicated by a ratio of 1.26:1. In the control group, the percentage of females with lymphomas/leukaemias (12.6%) was comparable to the historical control mean but it was three times higher than the lowest value for historical controls (4.0%), and 1.4 times higher than the value in the control group in the first ERF study. The Panel further noted that the percentage of male rats with lymphomas/leukaemias in the high-dose group was well within the incidence range for the historical controls in the ERF laboratory (range: 8.0-30.9%, data collected over the last 20 years), and that the incidence of lymphomas/leukaemias in the concurrent control group was within the lower range of the ERF historical controls.

Furthermore, it is uncertain which tumour types were included in the classification of lymphomas and leukaemias. The incidence and distribution of histotypes were not given, and an exact histopathological description of these tumours was not provided in the paper (Soffritti *et al.*, 2007). In the previous carcinogenicity study with aspartame by Soffritti *et al.* (2005; 2006; Soffritti and Belpoggi, 2005) myeloid leukaemias and histiocytic sarcomas were included in the total incidence of lymphomas and leukaemias for statistical purposes. The Panel concurs with the previous opinion of the AFC Panel that these types of tumours are of different cellular origin and for interpretation of results should not be combined with the lymphomas but treated as separate malignancies (EFSA, 2006). Thus, if aggregation of the haemolymphoreticular tumour types, involving a combination of tumours of different cellular origin, which is not justified in the view of the Panel, was performed for the statistical analyses of the 2nd ERF study, this might have influenced the outcome of the results.

In addition, it is well established that some lymphoreticular tumours in rats may occur as a consequence of chronic respiratory disease/chronic inflammatory changes in the lung (Innes *et al.*, 1967, Nelson, 1967, Swaen and van Heerde, 1973). The lack of data on such non-neoplastic changes prevents the interpretation of the results on the incidence of lymphomas/leukaemias in the present study.

5.4. Mammary carcinomas

The Panel noted that, as with the other tumours reported in this study, the increased incidence in mammary gland carcinomas in the high-dose group was reported only for data presented as percentages. The incidence of mammary gland carcinomas in the high-dose group (15.7%) was above the upper value for historical controls (range: 4.0-14.2%, data collected over the

last 20 years) but the increase was slight, as indicated by a ratio of 1.11:1, and the incidence of mammary gland carcinomas in the concurrent control (5.3%) was within the lower range of incidence for historical controls. While considering the biological significance of the slight increase in incidence of mammary gland carcinomas in the high-dose females, the Panel further noted that these tumours commonly occur at a rather high incidence in ageing female rats and show a highly variable incidence between studies (Greaves, 2000).

The Panel also noted that a statistically increased incidence of mammary gland carcinomas was not reported in the previous Soffritti *et al.* (2005; 2006) study even though much higher doses of aspartame were used.

6. Comparison of findings in the present and the previous ERF study on aspartame

6.1. Comparison of the incidence of lymphoma/ leukaemias in female rats

According to Soffritti *et al.* (2007), when comparing lifespan exposures beginning during prenatal and postnatal life, the prenatal exposure to aspartame clearly increases the incidence of lymphomas/leukaemias in females and accelerates the appearance of these lesions as indicated in both studies by the cumulative prevalence by age at death of animals with haemolymphoreticular neoplasias.

In its evaluation, the Panel concurs with Soffritti *et al.* (2007) that the reported percentage of females bearing lymphomas/leukaemias in the high-dose group in the present ERF study is higher than in the female group exposed postnatally to the same dose in the previous ERF study (Table 3).

Table 3. The incidence of lymphomas/leukaemias in female Sprague Dawley rats exposed to the same dietary doses of aspartame starting either post- or pre-natally.

Dose mg/kg bw	Previous ERF study (postnatal exposure, Soffritti *et al.*, 2006)		Present ERF study (prenatal exposure, Soffritti *et al.*, 2007)		Ratio[b)]
	% female rats bearing lymphomas /leukaemias	Ratio[a)]	% female rats bearing lymphomas /leukaemias	Ratio[a)]	
0	8.7	-	12.6	-	1.4:1
20	20.0	2.3:1	17.1	1.4:1	0.9:1
100	18.7	2.2:1	31.4	2.5:1	1.7:1

[a)]: Incidence in the treated group/incidence in the concurrent control.
[b)]: Incidence in the present study/incidence in the previous ERF study

However, the Panel noted, that the ratio between the incidence in the low-dose group and the incidence in the concurrent control is considerably lower in the animals exposed prenatally (1.4:1) compared to those exposed postnatally (2.3:1). The ratio in the groups exposed to 100 mg/kg bw/day relative to the respective concurrent controls is only slightly higher in animals exposed prenatally (2.5:1) compared to those exposed postnatally (2.2:1). The Panel also noted that the incidence of lymphomas/leukaemias in the control group in the study with prenatal exposure is higher than the control value in the previous study (ratio of 1.4:1), and the ratio between the incidence in the 100 mg/kg bw/day group in the present study relative to

that in the previous study is 1.7:1. The latter may indicate that the spontaneous incidence of lymphomas/leukaemias either increases with time in the breeding colony of Sprague Dawley rats in the ERF laboratory or is highly variable, which could make the toxicological data involving these tumour type difficult to interpret.

Similarly, consideration of the cumulative prevalence of death by age in female rats bearing lymphoreticular neoplasias (Figure 2 in Soffritti *et al.*, 2007) does not provide evidence that the prenatal exposure to aspartame accelerates the appearance of these lesions.

6.2. Other neoplastic changes reported in the life-long aspartame carcinogenicity study with postnatal exposure

In the previous life-time study with aspartame, Soffritti *et al.* (2005; 2006; Soffritti and Belpoggi, 2005) reported, in addition to induction of lymphomas/leukaemias, increased incidence of transitional cell carcinomas of the renal pelvis and ureter and their precursors (dysplasias), with a positive significant trend in female rats, and malignant schwannomas of peripheral nerves, with a positive significant trend in male rats.

As no data on these tumours are reported in the present study, it is unclear if these malignancies were also found in the present study.

7. Discussion

The rat study carried out by the ERF to further evaluate the carcinogenic effect of aspartame (Soffritti *et al.* 2007) was a lifespan study with prenatal exposure to low dietary doses of aspartame. The authors reported an increased incidence of animals with lymphomas/leukaemias, an increase in the total number of malignant tumours in treated animals and an increased incidence of females bearing mammary carcinomas compared to controls. The authors of the study conclude that these results confirm their previous findings (Soffritti *et al.*, 2006, Soffritti and Belpoggi, 2005) that aspartame is a carcinogenic compound. The Panel does not share this view based on the following arguments:

1. While the incidence of lymphomas/leukaemias as presented by the authors of the ERF study were statistically significantly increased in the high-dose groups (100 mg/kg bw/day) of both sexes when presented as percentages, the variation in the incidence of lymphomas/leukaemias in this strain of rats appears to be high. Additionally, most of the lymphomas were found in the lungs and peripheral lymph nodes, and, for females, also in the thymus and spleen. This leads the Panel to assume, as also highlighted by the AFC Panel in its evaluation of the first ERF life-long study, that inflammation of the lungs may have played a role in the etiology of lymphomas and leukaemias in the present study. However, it was not possible to further conclude on this hypothesis, since in the present study, data on the incidence and type of chronic inflammatory changes were not provided by the authors.

   Additionally, in the first carcinogenicity study with aspartame by Soffritti et al. (2005; 2006; Soffritti and Belpoggi, 2005), myeloid leukaemias and histiocytic sarcomas were included in the total incidence of lymphomas and leukaemias for statistical purposes. In the opinion of the Panel these types of tumours are of different cellular origin and should not be combined with the lymphomas but treated as separate malignancies as was previously stated by the AFC Panel in its review of the first ERF Study on aspartame (EFSA, 2006). This aggregation of the haemolymphoreticular tumour types, involving a combination of tumours of different cellular origin, if

performed for statistical purposes also in this present ERF study, might have influenced the outcome of the statistical analysis.

2. In the view of the Panel, the statistically significantly increased incidence of male rats with malignant tumours cannot be considered to be an indication of the carcinogenic potential of aspartame administered prenatally. The Panel noted that the percentage of tumour-bearing males in the high-dose group (40.0%) in the present study was comparable with the control value (39.3%) from the first ERF life-long study with postnatal exposure to aspartame, which may indicate high variability in spontaneous tumour development in the Sprague Dawley rats from the ERF breeding colony. In the view of the Panel, aggregation of malignant tumour incidences to support evidence of a carcinogenic potential of a test compound can only be performed when it is based on a thorough consideration of all the tumour data, including their onset, and data on preneoplastic lesions, hyperplastic and non-neoplastic changes. Such data were not provided in the Soffritti *et al.* (2007) publication.

   The relevance of the various tumours reported in the study for humans should be considered within a hazard assessment process. After hazard identification, the tumours considered to be of no relevance for humans or as incidental findings should be eliminated when aggregation of all malignant tumour incidences or total number of malignant tumours for statistical purposes is performed as part of the hazard characterisation.

3. Soffritti *et al.* (2007) considered the statistically significantly increased incidence of mammary gland carcinomas in the high-dose female group as indicative of a carcinogenic effect of aspartame. However, this incidence (15.7%) was only slightly higher than the upper value for historical controls (14.2%) but close to triple the value in the concurrent control (5.3%), which was at the low-end of historical controls (4%). The Panel noted that this type of tumour occurs at a rather high incidence in female rats and a large variation in incidence has been reported in different carcinogenicity studies. Therefore, the Panel considers it unlikely that the increased percentage of females bearing mammary gland carcinomas in the high-dose group was due to aspartame exposure. The Panel also noted that an increased incidence of mammary gland carcinomas were not reported in the previous ERF study with aspartame in which much higher doses of the sweetener were used. Consequently, this finding should not be considered as an indication of the carcinogenic potential of aspartame.

CONCLUSIONS

The Panel has assessed the recent publication of the ERF life-long study with prenatal dietary exposure to aspartame (Soffritti *et al.*, 2007) and has noted the authors conclusions, that the results of their study not only confirmed but also reinforced their first experimental demonstration of aspartame's multipotential carcinogenicity at a dose level close to the established ADI of 40 mg/kg bw/day, and that the study results also demonstrated that when a lifespan exposure to aspartame begins during fetal life, its carcinogenic effect is increased.

The Panel concluded that:

- Evaluation of aggregated malignant tumour incidences as evidence of carcinogenic potential of the test compound can only be performed based on a thorough consideration of all tumour data including onset, and data on non-neoplastic,

hyperplastic and preneoplastic lesions but these data were not provided by the authors by the time of the adoption of this opinion.

- In accordance with the previous hypothesis of the AFC Panel, the lymphomas and leukemias might have developed in a population of rats suffering from chronic respiratory disease.

- The increase in incidence of mammary gland carcinomas is not considered indicative of a carcinogenic potential of aspartame since the incidence of mammary tumours in female rats is rather high and varies considerably between carcinogenicity studies. The Panel also noted that an increased incidence of mammary gland carcinomas was not reported in the previous ERF study in which much higher doses of aspartame were used.

Overall, the Panel concluded, on the basis of all the evidence currently available from the results published from the ERF studies and previous evaluations, that there is no indication of any genotoxic or carcinogenic potential of aspartame and that there is no reason to revise the previously established ADI for aspartame of 40 mg/kg bw/day.

DOCUMENTATION PROVIDED TO EFSA

No documentation has been provided.

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GLOSSARY / ABBREVIATIONS

ADI	Acceptable Daily Intake
AFC	Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
ANS	Scientific Panel on Food Additives and Nutrient Source added to Food
APM	Aspartame
bw	body weight
EC	European Commission
EFSA	European Food Safety Authority
ERF	European Ramazzini Foundation
GLP	Good Laboratory Practice
OECD	Organisation for Economic Co-operation and Development
SCF	Scientific Committee on Food