COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

STATEMENT ON A CARCINOGENICITY STUDY OF ASPARTAME BY THE EUROPEAN RAMAZZINI FOUNDATION

COC/06/S2 – December 2006

Introduction and background

1. Aspartame is a widely used artificial sweetener which was initially approved in 1982 and has been reviewed on several occasions subsequently.

2. In July 2005, a carcinogenicity study conducted by the independent European Ramazzini Foundation of Oncology and Environmental Sciences (ERF) (Soffritti *et al.* 2005) was published as part of their research programme. This suggested that aspartame was associated with an increase in lymphomas and leukaemias in male and female rats. The COC considered the publication briefly in July 2005 and expressed a number of concerns about the design and conduct of the study. A second more detailed paper was then published (Soffritti *et al.,* 2006). This study reported increases in pre-neoplastic and neoplastic lesions of the renal pelvis and ureter, malignant schwannomas of peripheral nerves and pre-neoplastic and neoplastic lesions of olfactory epithelium as well as the findings in leukaemias and lymphomas. An increase in the total load of malignant tumours was also reported. The main contributors to the overall tumour load were lymphomas and leukaemias.

3. Following a request from the European Commission, the European Food Safety Authority (EFSA) Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) reviewed the findings. EFSA requested and received the full study report (Soffritti and Belpoggi, 2005) and undertook a full evaluation of the study in the context of previous safety data. As part of this process, the Food Standards Agency sought the views of the COC again in March 2006 on the quality of the study and its implications for interpretation of the results.

4. Following an initial consideration of the published papers and the unpublished study report, the committee requested clarification of a number of points and further data through EFSA. Some additional information supplied by the ERF was considered at a subsequent meeting.

Background on aspartame

5. In the UK, aspartame was initially approved for use in 1982 as category A, a substance “that the available evidence suggests are acceptable for use in food” (FACC, 1982) with data on metabolism, short and long term

toxicity, carcinogenicity, mutagenicity and reproduction studies being received as part of the manufacturer's submission. A detailed review of aspartame was undertaken by the Committee on Toxicity (COT) in 1992 and an Acceptable Daily Intake (ADI) of 40 mg/kg bw/day established. As new data have been published, aspartame has been reconsidered by expert committees in the UK and the EU on a number of occasions, most recently in 2002 by the EU Scientific Committee on Food (SCF, 2002) when it was concluded that it was unnecessary to revise the previous risk assessment or ADI.

The study by the European Ramazzini Foundation (ERF)

6. In the ERF study, Sprague-Dawley rats from an in-house colony were fed pelleted diets containing 0, 80, 400, 2000, 10,000, 50,000 or 100,000 ppm aspartame from 8 weeks of age until natural death. The received doses of aspartame were not measured but were estimated to be 0, 4, 20, 100, 500, 2,500 or 5,000 mg/kg bw/day over the course of the experiment.

7. A selection of the pathology slides were sent to a working group of pathologists from the US National Toxicology Program (PWG) however it was noted in the PWG report that this could not be considered a peer review (Hailey, 2004). The findings of the PWG are discussed in detail by EFSA (EFSA, 2006).

8. The results were compared to historical control data from studies conducted in the laboratory over the previous twenty years, comparing the results from groups of 100 or more animals and from groups using fewer than 100 animals.

Results

9. Overall, a significant increase in malignant tumour- bearing animals of both sexes was reported. The individual tumour types are considered in more detail below.

Lymphomas and leukaemias

10. A statistically significant dose-related increase in lymphomas was observed in the females given 400 ppm or more aspartame compared to the controls. An increase was also apparent in the 80 ppm females and the top dose males compared to the controls but was not statistically significant. A positive trend test was reported for males and females ($p \leq 0.05$ and 0.01 respectively). The haemolymphoreticular neoplasias observed included lymphoblastic lymphoma and leukaemia, lymphocytic leukaemia, lymphocytic lymphoma, lymphoimmunoblastic lymphoma, histiocytic sarcoma and monocytic leukaemia. The most frequent type of neoplasia was the lymphoimmunoblastic lymphoma. The overall incidence of lymphomas and leukaemias was:

Aspartame (ppm)	0	80,	400,	2000	10,000	50,000	100,000

Females (%)	8.7	14.7	20.0	18.7	19.0,	25.0	25.0
Males (%)	20.7	15.3	16.7	22.0	15.0,	20.0	29.0

Controls, 80, 400 and 2000 ppm aspartame; 150 animals/group.
10,000, 50,000 and 100,000 ppm aspartame; 100 animals/group

11. Historical control data from groups of 100 or more animals showed an overall incidence of lymphomas and leukaemias of 20.7% in the males (8-30.9%) and 12.4% (7-18. 4%) in the females (the mean incidences given in Soffritti *et al* (2005a) differ very slightly). The results suggest that the incidence in the treated females was above the historical range in the top fivedose groups, but within the historical range for the males. Historical data from groups of fewer than 100 animals were provided and were comparable.

Pre-neoplastic and neoplastic lesions of the renal pelvis and ureter

12. A dose-related increase in the incidence of dysplastic hyperplasia and dysplastic papilloma of the transitional cell epithelium of the renal pelvis was observed in the treated females. Carcinomas occurred in females with a positive trend ($p \leq 0.05$) and were significantly increased ($p \leq 0.05$) at the top dose compared to the controls. Carcinomas were also observed in the males receiving 2000 ppm or more aspartame but this was not dose-related. When dysplastic lesions and carcinomas were combined, a significant positive trend was apparent in females ($p \leq 0.01$) and the incidence of the lesions was significantly increased at levels of 2000 ppm and above. The combined incidence was:

Aspartame (ppm)	0	80,	400,	2000	10,000	50,000	100,000
Females (%)	1.3	4.0	6.0	6.7	10.0	10.1	15.0
Males (%)	0.7	2.0	3.4	3.3	3.0	3.0	4.0

Controls, 80, 400 and 2000 ppm aspartame; 150 animals/group.
10,000, 50,000 and 100,000 ppm aspartame; 100 animals/group

13. It was noted that transitional cell carcinomas of the renal pelvis and ureter were very rare in rats and had only been found in the treated animals. The historical control data indicated that these had not occurred previously in groups of more than 100 animals but had an incidence of 0.04% (0-1.0%) in groups of fewer than 100 female controls only.

Malignant schwannomas of peripheral nerves

14. There was an increased incidence of malignant schwannomas of peripheral nerves with a positive trend in males ($p \leq 0.05$). The most frequent site of origin was in the cranial nerves. The incidence was:

Aspartame (ppm)	0	80,	400,	2000	10,000	50,000	100,000

Females (%)	0	1.3	0	2.0	1.0	1.0	2.0
Males (%)	0.7	0.7	2.0	1.3	2.0,	3.0	4.0

Controls, 80, 400 and 2000 ppm aspartame; 150 animals/group.
10,000, 50,000 and 100,000 ppm aspartame; 100 animals/group

15. Historical control data from groups of more than 100 animals indicated an incidence of 0.5% (0-2%) in males and 0.1% (0-1%) in females. The historical control data from groups of fewer than 100 animals were comparable

Pre-neoplastic and neoplastic lesions of the olfactory epithelium

16. An increase in hyperplasia of the olfactory epithelium with a significant positive trend test was observed in males and females ($p \leq 0.01$). This was characterised by increased thickness of the epithelium. The observed incidences were:

Aspartame (ppm)	0	80,	400,	2000	10,000	50,000	100,000
Females (%)	4.0	3.3	7.3	8.7	17.0	21.0	19.0
Males (%)	0.7	2.0	6.0	2.7	7.0,	12.0	14.0

Controls, 80, 400 and 2000 ppm aspartame; 150 animals/group.
10,000, 50,000 and 100,000 ppm aspartame; 100 animals/group.

17. The differences were statistically significant compared to the controls at levels of 10,000 ppm and above in males and females ($p \leq 0.01$) and also in males given 400 ppm aspartame. Historical control data from groups of both more than and fewer than 100 animals showed an overall incidence of 0.1% (0-1.8%) in males and females

Other lesions

18. Malignant brain tumours were observed in the treated animals but none in the controls. The reported incidence was 0.7-1% in the females and 1-2% in the males with no clear dose-response. The historical incidence for the lesions was 1.7% (0-5%) in the males and 0.7% (0-2%) in the females.

19. Other malignant tumours observed were those commonly found in Sprague-Dawley rats, with the exception of 2 transitional cell carcinomas of the bladder in 10,000 ppm males and 1 in a 2000 ppm female. These had not been observed in the historical controls.

20. The authors suggested (Soffritti *et al* 2005) that the lymphomas and leukaemias may be related to the formation of methanol and subsequently formaldehyde during the metabolism of aspartame. They noted that their previous studies have shown that methanol in drinking water, methyl-*tert*-

butyl ether (which is metabolised to methanol) and formaldehyde were also associated with an increase in lymphomas and leukaemias.

21. In addition to the mechanisms discussed in the initial paper, the authors speculated (Soffritti *et al* 2006) that aspartic acid may be responsible for the lesions observed in the renal pelvis and ureter, proceeding via calcification which was observed in treated females but not in the controls or the males.

22. Overall, the authors concluded that aspartame was a multi-potential carcinogenic agent even at doses of 20 mg/kg bw/day. They noted that this contrasted with the results of previous bioassays and considered this to be due to the larger group size and because the animals were observed until they died naturally rather than being culled at 110 weeks of age, allowing the aspartame to express its full carcinogenic potential. It was also suggested that the Wistar rats used in other studies could be more resistant.

<u>COC discussion</u>

Conduct of the study

23. The study was stated to have been conducted in accordance with the principles of Good Laboratory Practice (GLP). However, whilst certain aspects of GLP may have been incorporated into the design, there was no external quality control which is required for GLP compliance.

24. The test material used was food grade aspartame supplied by the manufacturers and meeting the specifications for aspartylphenylalanine diketopiperazine and free phenylalanine; this was checked by infra-red absorption spectroscopy (EFSA, 2006). Thus the only purity assessment of the test material used was qualitative and, therefore, inadequate. The stability of the test material in the diet had not been assessed, which would usually be standard procedure in a rodent carcinogenicity study. Given that some of the dietary dose levels were very high, the possibility that an impurity or degradation product was responsible for the observed pathology could not be excluded. The high dietary doses may also have resulted in a nutritional imbalance in the top dose groups.

25. The ERF report states that the tissue samples were fixed in 70% ethyl alcohol. COC considered that, if correct, this would be likely to dehydrate the samples, rendering histopathological evaluation very difficult and possibly leading to errors.

26. Among the non-neoplastic effects reported were abscesses in the brain, the incidence ranging from 4-20% in the different treatment groups. Bronchopneumonia was observed in 69-96% of the animals in the various treatment groups and pleuritis in 22-94%. This suggested that there might have been a high level of mycoplasma infection within the rat colony.

Mycoplasmosis is a lymphocyte mitogen and this may be the explanation for many of the lymphomas which were found in the lung. It was unclear from the study report whether any screening for infection had been carried out. The study report commented that the bronchopneumonia may have contributed to the spontaneous death of both test and control animals. The NTP PWG had also noted the poor animal health in the ERF study.

27. Members also noted there were differences in interpretation between the NTP PWG's peer review and the original histological diagnosis reported by the Ramazzini Foundation. In general, the ERF tended towards a more severe diagnosis of the lesion than the PWG.

28. The COC considered that comparison of the study results with historical control data from a 20 year period was not valid. Comparison with historical control data from the previous 5 years is considered more appropriate because of the genetic drift in tumour incidence. Historical control data from experiments starting in the period 1984-1991 were subsequently supplied. These indicated similar tumour incidences to the initial historical data. However, it is unclear whether these data were for studies in which the rats were sacrificed after 2 years, living a natural lifespan or a combination of the two. The ERF aspartame study began in June 1997.

29. The animals were allowed to reach a natural death rather than sacrificing them at the same time point. Whilst it was noted that care was taken to minimise post-mortem changes, autolytic changes were noted (discussed EFSA, 2006). The differences in lifespan were adjusted for statistically by using the poly k test which, although not commonly used, has been recommended by the US NTP. However it is usually applied when the animals have been sacrificed at different time points rather than living a natural lifespan. In general, the groups fed with aspartame had lower body weights and thus lived longer, which may have compromised the results since this may lead to an apparent increase in spontaneously arising tumours.

Findings of the study

30. Dysplasia and carcinoma of the transitional cell epithelium of the renal pelvis may be related to the calcification also observed. A link has previously been established between calcification and transitional cell carcinoma. The findings in the renal pelvis could also be due to urinary tract infection.

31. Schwannoma is not an uncommon finding in carcinogenicity studies. As with the other reported malignancies, the dose response relationship for this finding is very shallow. It is also worth noting that the stains used to diagnose schwannomas are a relatively recent development and so the results of the most recent study may not be comparable to historical data.

32. It is not appropriate to add together all the malignant tumours in the reporting or analysis of results nor to combine the numbers of lymphomas and leukaemias.

Overall Conclusions

33. In view of the inadequacies in design of the ERF study and the use of rats with a high concurrent infection rate, the COC considered that no valid conclusions could be derived from it.

34. The committee agreed with the evaluation of the EFSA panel, which published its review of the data in July 2006, that this study did not indicate a need for a review of the ADI for aspartame**.**

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REFERENCES

COT (1992). 1992 Annual report of the Committees on Toxicity, Mutagenicity, Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. The Stationary Office, London.

EFSA (2006). Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) on a request from the Commission related to a new long-term carcinogenicity study on aspartame. Question number EFSA-Q-2005-122. The EFSA Journal (2006) 356, 1-44. http://www.efsa.eu.int/science/afc/afc_opinions/1471_en.html

FACC (1982) Food Additives and Contaminants Committee, Report on the Review of Sweeteners in Food. FAC/REP/34. HMSO, London.

Hailey, J. R. (2004). Pathology Working Group Chairperson's Report. Lifetime Study in Rats Conducted by the Ramazzini Foundation. Prepared by James R Hailey, Pathology Working Group Chair. National Institute of Environmental Health Sciences, Research Triangle Park, USA. Submitted to: Dr Fiorella Belpoggi, Ramazzini Foundation, Bologna, Italy.

SCF (2002). Opinion of the Scientific Committee on Food: Update on the Safety of Aspartame SCF/FS/ADD/EDUL/222Final expressed on 10th December 2002.

Soffritti, M. and Belpoggi, F. (2005). Long-term carcinogenicity Bioassay to Evaluate the Potential Biological Effects, in Particular Carcinogenic, of Aspartame Administered in the Feed to Sprague-Dawley Rats (Protocol no: BT 6008). Unpublished report of the European Foundation of Oncology and Environmental Sciences "B Ramazzini", December 2005 Bologna, Submitted to EFSA.

Soffritti, M., Belpoggi, F., Degli Esposti, D., Lambertini, L. (2005). Aspartame induces Lymphomas and Leukaemias in rats. European Journal of Oncology, 10, 107-116.

Soffritti, M., Belpoggi, F., Degli Esposti, D., Lambertini, L., Tibaldi, E., Rigano, A. (2006). First Experimental Demonstration of the Multipotential Carcinogenic Effects of Aspartame Administered in the Feed to Sprague-Dawley Rats. Environmental Health Perspectives, 114, 379-385.