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

RAMAZZINI STUDY ON THE CARCINOGENICITY OF ASPARTAME.

Introduction

1. Aspartame (see Fig 1) is a widely used artificial sweetener which was initially approved in 1982 and has been reviewed on several occasions subsequently. It was most recently reviewed in 2002 by the Scientific Committee for Food (SCF) who considered the data available from 1989 onwards and concluded that there was no need to revise the previous risk assessment or Acceptable Daily Intake.

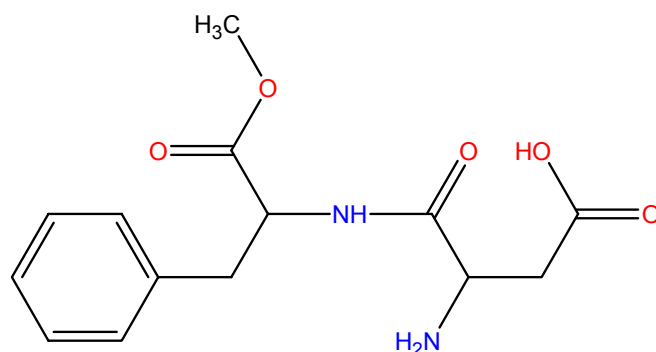


Figure 1. Aspartame (6-methyl-1,2,3-oxathiazine-4(3H)-one-2,2-dioxide salt of L-phenylalanyl-2-methyl-L- α -aspartic acid).

2. In July 2005, a carcinogenicity study by Soffritti *et al.* (2005a) was published which suggested that aspartame was associated with an increase in lymphomas and leukaemias in rats. The COC considered the publication and expressed a number of concerns about the study at the July meeting (see below). A second paper was published in November 2005 (Soffritti *et al.*, 2005b).

3. The European Food Safety Authority (EFSA) requested and received the full study report. EFSA will be undertaking a full evaluation of the study in the context of previous safety data. As part of this process the Food Standards Agency is seeking the views of the Committee on the quality of the study and its implications for interpretation of the results. The views of the Committee will be passed on to EFSA to assist in their detailed consideration.

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Background

4. Aspartame was initially approved for use in the UK in 1982 (FACC, 1982). A dossier was initially submitted by the manufacturers in 1974, but concerns about the conduct of the studies delayed approval until 1982. Data on metabolism, short and long term toxicity, carcinogenicity, mutagenicity and reproduction studies were submitted as part of the package. Aspartame was classified as Group A, “substances that the available evidence suggests are acceptable for use in food”. A full review of aspartame was undertaken by the Committee on Toxicity (COT) in 1992. The review considered several issues including the potential risk to health in light of surveyed levels of intake in both the general population and diabetics. The COT also assessed scientific data from the literature published after the initial approval and established an ADI of 40 mg/kg bw/day for aspartame rather than using category A or B status.

5. The COC last considered aspartame in 1996, following the publication of a paper by Olney *et al* (1996) which linked aspartame to an increased incidence of brain tumours in humans. The COC concluded (COC, 1996) that the data presented and methods used by Olney *et al* were misleading and did not raise concerns with regard to the use of aspartame in the UK.

6. Aspartame was most recently reviewed in 2002 by the SCF (SCF, 2002). This review included extracts from a detailed assessment of the suggested link between aspartame and brain tumours which had been carried out by AFSSA (Agence Française de Sécurité Sanitaire des Aliments, Maisons-Alfort). The AFFSA review examined all the relevant data including studies on carcinogenicity and genotoxicity. Both AFSSA and SCF concurred with the COC view that there was no evidence for such a link and that it was unnecessary to revise the previous risk assessment or ADI. The SCF opinion and a 1990 JECFA review (WHO, 1990) are attached at Annex A, and provide additional background information.

7. The study conducted by the Ramazzini foundation (Soffritti *et al*, 2005a) was published in July 2005 (see Annex B) and was tabled for discussion under any other business at the 14 July COC meeting. The agreed minutes were as follows:

- *An increased incidence of lymphomas and leukaemias was reported in rats. There are slight differences in historical control data for the incidences of lymphomas and leukaemias in female rats between the published data (average 13.4%, range 7.0-18.4%) and data presented to the EFSA on 17 June 2005 (average 12.9%, average 4-25%). The incidences of lymphomas and leukaemias in female aspartame-fed rats occurred over the range of the historical control data sets presented in the paper. Members considered that a small increase a tumour incidence over such a wide range of doses was implausible. Members agreed that there may be reasonable explanation for the differing*

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historical control figures but that the variation in control data casts doubt on the quality of the study observations. Members questioned the validity of the practice of allowing the rats to live until a natural death. It was noted the statistical approach used did not fully adjust for age related effects.

8. A more detailed version of the study was published subsequently on-line in November 2005 (Soffritti *et al.*, 2005b) (see Annex C). This paper acknowledges the assistance of the US National Toxicology Program (NTP) which convened a group of pathologists at NIEHS to “provide a second opinion for a set of lesions of malignancies and their precursors related to aspartame treatment and for the help in statistical analysis”. The paper expands on the initial paper to report on tumours in the kidney, nervous system and olfactory epithelium.
9. The full study report including the individual animal data has been supplied to EFSA and is contained in the attached CD with the summary section of the report being attached at Annex D.

Ramazzini Study

10. The National Ramazzini Institute for the Study and Control of Tumours and Environmental Diseases is a non-profit association. The institute undertakes a variety of activities including research, training, outpatient and hospice care. The Institute was founded in 1987 and is a social co-operative with over 14,000 members. It has the stated aim of protecting health, the environment and its resources. It has a strategy of research and control over cancer and other environmental diseases and supports an integrated programme of projects against cancer (Ramazzini Institute, 2006). The research program is conducted by the European Ramazzini Foundation for Oncology and Environmental Sciences which was founded by the National Ramazzini Institute in 1992.
11. The details below are taken from the Soffritti *et al.* (2005a and b) and the full study report.

Method

12. The study was conducted according to in-house quality control procedures referred to in the report as according to Good Laboratory Practice (GLP). Sprague-Dawley rats from an in-house colony were fed aspartame in the diet at 0, 80, 400, 2000, 10,000, 50,000 or 100,000 ppm from 8 weeks of age until natural death. The control, 80, 400 and 2000 ppm groups contained 150 animals of each sex whereas the higher doses had 100 animals of each sex. The average doses of aspartame were estimated to be 0, 4, 20, 100, 500, 2,500 or 5,000 mg/kg bw/day over the course of the experiment, assuming an average body weight of 400g and food intake of 20 g/day. This was calculated to be 0, 0.08, 0.4, 2, 10, 50 or 100 times the human ADI of 50

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mg/kg bw/day (as set by the US FDA). Statistical analysis was undertaken using the Cochrane-Armitage trend test to test for linear trends and the poly-k test (k=3) which modifies the Cochrane-Armitage linear trend test to take survival differences into account.

13. It was considered most appropriate to use historical control data from studies conducted in the previous 20 years at the Institute where group sizes of 100 or more animals were used. This represents 1934 and 1945 male and females respectively. It is unclear what proportion of the historical controls were animals that had been allowed to reach a natural death. Historical control data are also presented from studies with less than 100 animals/group representing 2265 male and 2274 female rats. The number of individual treatment groups this represents is not stated.

Results

14. Mean water consumption was unaffected by treatment other than a slight decrease in the 100,000 ppm groups compared to the controls from 72 weeks onwards. Mean food consumption was slightly decreased in all the treated groups compared to the controls. This appears to be dose-related but a statistical analysis is not presented. Body weights were not “substantially” affected, though it is noted that compared to the controls a decrease was apparent at the top dose, particularly in the females (see tables 6 and 7, and figs 7 and 8 of Annex D). Statistical analysis of these data is not presented, however, there is no clear trend apparent and mean group body weights are generally within 10% of the controls.

15. It was noted that “no substantial differences” in survival were apparent in males. Survival was noted to be slightly decreased in the control females from 104 weeks onwards (for example, 27.3% survival in the control females compared to 45% survival at 100,000 ppm after 103 weeks of treatment). The last animal had died by 155 weeks (males) and 159 weeks females) (see tables 8 and 9, and figs 7 and 8 of Annex D). Statistical analysis of these data is not presented.

16. The study reported an increased incidence of malignant tumour-bearing animals, with a positive significant trend in males and females ($p \leq 0.05$ and $p \leq 0.01$ respectively) (see Tables 14 and 15 of Annex D). The incidence of malignant tumours was significantly increased ($p \leq 0.01$) in females of the 50,000 ppm group compared to the controls. The main contributors to the overall load were lymphomas and leukaemias, 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. The number and percentage of animals bearing particular types of tumour are presented in Tables 11-12 of Annex D)

Lymphomas and leukaemias

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17. 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 (see Table 22 of Annex D) 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

18. 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 female (the mean incidences given in Soffritt *et al* (2005a) differ very slightly). The results suggest that the incidence in the treated females was above the historical range in the top 4 dose groups, but within the historical range for the males. Historical data from groups of less than 100 animals are provided and are comparable (see para 3.9, page 18 of the study report in Annex D).

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

19. 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 (Tables 16-18, Annex D). Carcinomas in females occurred 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

20. It was noted that transitional cell carcinomas of the renal pelvis and ureter are very rare in rats and were only found in the treated animals.

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21. 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 less than 100 female controls only.

Malignant schwannomas of peripheral nerves

22. An increased incidence of malignant schwannomas of peripheral nerves with a positive trend in males was observed ($p \leq 0.05$) (Table 21, Annex C). 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

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

Pre-neoplastic and neoplastic lesions of the olfactory epithelium

23. An increase in hyperplasia of the olfactory epithelium with a significant positive trend test in males and females ($p \leq 0.01$) was observed, 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

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.

24. Historical control data from groups of both more than and less than 100 animals showed an overall incidence of 0.1% (0-1.8%) in males and females.

Other lesions

25. 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.

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26. 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.

27. The authors suggested (Soffritti *et al* 2005b) that the lymphomas and leukaemias may be related to the formation of methanol and subsequently formaldehyde during the metabolism of aspartame and note that their previous studies have shown that methanol in drinking water, methy-*tert*-butyl ether (which is metabolised to methanol) and formaldehyde are also associated with an increase in lymphomas and leukaemias.

28. In addition to the mechanisms discussed in the initial paper, the authors speculated (Soffritti *et al* 2005b) 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.

29. Overall, the authors concluded that aspartame is a multi-potential carcinogenic agent even at doses of 20 mg/kg bw. This was noted to contrast with the results of previous bioassays and was thought 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.

Discussion

30. The protocol used is unusual with the animals allowed to reach a natural death rather than being sacrificed at the same time point. This was adjusted for statistically. Many of the animals survived considerably longer than the 110 weeks of age at which carcinogenicity bioassays are generally terminated, the last one dying at 159 weeks. Towards the end of the study (71 weeks of treatment onwards) there appears to be a dose-related trend towards increased survival in the females which becomes more marked as the study progresses. However, the longest living animals of both sexes tended to be in the mid dose treatment groups, rather the controls or high dose treatment groups. The authors claimed that terminating bioassays at 110 weeks of age could lead to failure to demonstrate carcinogenic effects.

31. The range of concentrations used was very wide, from 80-100,000 ppm in the diet (estimated by the authors to be equivalent to 4 to 5000 mg/kg bw/day). The dose response is very shallow given this range. The top dose of 100,000 ppm (10%) is in excess of the OECD recommended 5% maximum for a diet mixture (OECD, 1998) and which suggests it might have compromised the nutritional status of the animals or resulted in otherwise spurious results. However the high dose does not appear to have affected body weights or resulted in significant toxicity as determined by clinical signs.

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It is uncertain what the effect on the statistical analysis would be if data from the 100,000 ppm treatment group were removed.

32. Some of the changes reported are within or close to the historical control range even at high doses, however, a number of the tumours observed are considered to be rare in controls.

33. Several of the tumours observed occurred more frequently in one sex, notably the lesions in the transitional cell epithelium of the renal pelvis and ureter which were much more frequent in the females. The incidence of lymphomas and leukaemias was much lower in the female controls (both in the experimental and historical controls, resulting in a statistically significant effect even though the incidences were comparable between males and females treatment groups. It seems likely that the achieved dose was higher in the females due to their lower body weight but this information is not provided.

34. A description and analysis of the diet is not provided but it is possible that the changes in the olfactory epithelium could be due to irritation from inhalation of aspartame.

Questions for the Committee

35. Do Members have any comments on:

- a) the quality of the study,
- b) the analysis of the data
- c) the interpretation of the results

Secretariat
February 2006

Note: Annexes A-C of this paper are not being made available since they contain full copies of references which cannot be distributed for copyright reasons but can be obtained from publicly available sources. Annex D of the paper is not being made available since it contains unpublished study data. This may be available from the National Ramazzini Institute for the Study and Control of Tumours and Environmental Diseases

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