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CC/06/17

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

**RAMAZZINI STUDY ON THE CARCINOGENICITY OF ASPARTAME –
FURTHER INFORMATION.**

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 by Soffritti *et al.* (2005) was published which suggested that aspartame was associated with an increase in lymphomas and leukaemias in male and female rats. The study was conducted by the independent European Ramazzini Foundation of Oncology and Environmental Sciences (ERF) as part of their research programme. The COC considered the publication in March 2006 and expressed a number of concerns about the study. A second more detailed paper was then published (Soffritti *et al.*, 2006). In addition to the increased incidence of lymphomas and leukaemias, increases in transitional cell carcinomas of the renal pelvis and ureter in females and malignant schwannomas of peripheral nerves in males and an increase in pre-neoplastic and neoplastic lesions of the olfactory epithelium were also reported. Overall, a significant increase in malignant tumour bearing animals of both sexes was apparent. This is considered in detail in CC/2006/06.
3. Following a request from the European Commission, the European Food Safety Authority (EFSA) requested its Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) to review the findings. EFSA requested and received the full study report 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 on the quality of the study and its implications for interpretation of the results at the March 2006 meeting.
4. The Committee expressed a number of concerns about the conduct of the study and requested additional information from the European Ramazzini Foundation (ERF) - see paragraphs 23-25 of the draft minutes. The preliminary views of the Committee were passed on to EFSA to assist in their detailed consideration.
5. EFSA also received additional information and completed their assessment in May 2006. Their review is attached at annex A. Overall they concluded that there was no need to revise the previous risk assessment or Acceptable Daily Intake.

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COC consideration of the Soffritti *et al* studies

6. At the March 2006 meeting, Members considered the carcinogenicity study by the ERF in detail.

7. A number of concerns were identified. These included the high level of infection within the colony, the high doses of aspartame and the potential for nutritional imbalance, the summation of certain tumour types in the analysis as well as certain methodological aspects of the study. Overall, Members concluded that in view of the problems in the design of the study and concerns about the microbiological status of the colony it was not possible to draw firm conclusions about the potential carcinogenicity of aspartame from the results.

8. The committee asked for further information to clarify a number of points.

- Clarification about the fixative used.
- An analytical specification for the test substance.
- Individual animal data, including data on pathology findings.
- The extent of the external histopathology review

9. The request for information was submitted via EFSA since it overlapped with additional information requested by the EFSA panel. The additional data received by EFSA has been shared with the COC. However, no direct response has been received from ERF. Each item is considered below

Fixative

10. In the original study report seen by the COC (Soffritti and Belpoggi, 2005) it was stated that the tissue fixative used (excepting bones) was 70% ethyl alcohol. Members considered that this could dehydrate the tissues, and may have been a translation error. No further information has been received from the ERF or submitted to EFSA. The use of the fixative is noted in a paper by Huff (2002) which compares the procedures used for bioassay by the US National Toxicology Program and the ERF.

Specification of the test substance.

11. The aspartame used was a food grade material supplied by Nutrasweet, with a specification for aspartylphenylalanine diketopiperazine of <1.5% and free phenylalanine of <0.5%. The purity was checked by infra red absorption spectroscopy (EFSA, 2006). The analytical data were not included in the study report (Soffritti and Belpoggi, 2005) and have not been provided subsequently. Stability data have not been provided.

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Pathology data

12. Information on pre-neoplastic and neoplastic lesions and the supporting individual animal data was considered in the previous paper CC/2006/06.
13. The non-neoplastic changes were summarised in Table 10 of the unpublished study report (Soffriti and Belpoggi, 2005) which is reproduced at Annex B). The changes include encephalic abscesses (4-20%), meningitis (7.3-19%) bronchopneumonia (81.3-96.7%) pleuritis (22-94%) pericarditis (5.3-27%) liver abscesses and hepatitis (2-20%) pyelonephritis (23.3- 83%) and peritonitis (3.3-28%). No clear dose-related changes are apparent. The report notes that acute and chronic inflammatory processes were the most common, particularly in the lungs and kidneys. It was stated that the high incidence of bronchopneumonia observed in both treated and control rats may have been related to the spontaneous death of animals.
14. The individual animal pathology data are contained in the attached CD. In addition to the findings noted above, statistically significant changes are reported in the following tissues in males:

Adrenals, bone (cranium), ear, epididymus, harderian gland, intestine (large and small), larynx, pancreatic islets, lymph nodes, mammary gland and duct, nose (sinus, olfactory epithelium), oral mucosae, pancreas, peritoneum, pharynx, pituitary gland, pleura, prostate, salivary glands, skeletal muscle, (diaphragm), skin (brown fat pad), spleen, forestomach, glandular stomach, testes, thymus, tissue not otherwise specified (mediastinum) trachea, thyroid, follicular cell of thyroid, tongue, zymbal's gland.

In the females, statistically significant changes in the following were reported:

Adrenals, bone (cranium), ear, internal ear, oesophagus, harderian gland and duct, intestine (large and small), pancreatic islets, larynx, lymph nodes mammary gland and duct, nose (sinus, olfactory epithelium), oral mucosae, ovary, pancreas, peritoneum, pharynx, pituitary gland, pleura, pancreas and duct, salivary glands, skeletal muscle (diaphragm), skin (subcutaneous tissue, spleen, forestomach, glandular stomach, testes, thymus, tissue not otherwise specified (mediastinum) trachea, thyroid, tongue, urinary bladder, zymbal's gland.

While the changes are noted to be statistically significant they are not generally dose-related. The majority of the non-neoplastic changes are inflammatory in nature.

15. At the previous COC meeting, it was suggested that the pre-neoplastic and neoplastic changes in the olfactory epithelium could be due to irritation arising from inhalation of particles of diet. A dose-related increase in

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hyperplasia of the olfactory epithelium is apparent in both males and females. This is not observed in the respiratory epithelium of the nose. Inflammation of the nose was observed but was not dose-related, a high incidence (up to 89%) of inflammation of the sinus was also observed, but no clear dose relation is apparent. At the March 2006 meeting, it was reported by one of the observers that the diet was in the form of pellets, but this has not been confirmed by the ERF.

Peer review

16. Pathology slides selected by the pathologist at ERF were sent to a working group of pathologists from the US National Toxicology Program (PWG). The PWG report (Hailey, 2004) stated that since the use of the results and discussion of the PWG were at the discretion of the Institute, the PWG report could not be considered a peer review. The PWG report is attached at Annex C. In relation to the four main findings reported by the ERF, the PWG had the following comments.

17. The diagnoses of lymphocytic and histiocytic neoplasma in the cases reviewed were generally confirmed. It was stated that the NTP does not generally sub-divide lymphomas into specific histological types as done by the ERF, however the PWG accepted the diagnosis if the lesion was considered to be consistent with a neoplasm of lymphocytic histiocytic monocytic and/or myeloid origin. The EFSA panel agreed with this view but considered that the sub division should only be done when necessary and possible. They considered it was also scientifically sound to aggregate lymphoblastic lymphomas, lymphocytic lymphomas, lymphoimmunoblastic lymphomas and lymphoblastic leukaemias as malignant lymphomas, but that myeloid leukaemias and histiocytic sarcomas (plus the one case of monocytic leukaemia) should be treated as separate malignancies and should not be combined with the lymphomas as they were of different cellular origin.

18. A few lesions diagnosed as atypical hyperplasia and neoplasia of the transitional epithelium of the renal pelvis were reviewed by the PWG. While there was general agreement that there was hyperplasia, and that at least some of the lesions were neoplastic, the general consensus of the PWG was that the use of the descriptor "atypical" was not warranted. In many instances there was pyelitis or nephritis or both in one or both kidneys and the proliferative lesions appeared to be associated with (secondary to) these inflammatory lesions. The EFSA panel noted that the ERF had used a more severe classification than the PWG and that since there were 24 reported carcinomas this difference in view could affect the outcome and interpretation of the study.

19. Two cases diagnosed as olfactory neuroblastoma were considered to be invasive malignancies by the PWG but more definitive classification would require the use of immunohistochemical stains. Interpretation of these stains may be complicated by post-mortem autolysis. The EFSA panel noted that the adenomas of the olfactory epithelium noted by the ERF might be hyperplasia

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since the two cases of early adenoma reviewed by the PWG were considered to be hyperplasia.

20. Cases diagnosed as malignant schwannomas of the cranial nerve were generally confirmed by the PWG. However, some members of the PWG felt it difficult to be definitive based on the H&E sections presented and preferred a diagnosis of sarcoma-NOS (not otherwise specified).

21. The EFSA panel considered that the more severe diagnosis used by the ERF pathologist compared to the PWG would affect the calculation of total-tumour bearing animals and thus that the data could not be used to assess the carcinogenic potential of aspartame.

Other Issues

Historical control data

22. The COC considered that the historical control data were not valid for comparison since went back 20 year period whereas it would be more normal to compare results with data from the previous 5 years. Historical control data from experiments starting in the period 1984-1991 have been supplied and are included in the attached CD and attached at Annex D.

23. The control incidence of lymphomas and leukaemias in the aspartame study was 8.7% in females and 20.7% in males. In the data supplied, the average was 13.32% in females (range 4-18.44%) and 20.62% in males (range 8-30.91%). Some data are give for breeders and offspring. This is taken from experiments where the animals were treated from gestational day 12 onwards and then maintained for the duration of the experiment. Tumours of the renal pelvis, malignant schwannomas of the peripheral nervous system and lesions of the neoplasms of the olfactory epithelium occur sporadically throughout the experiments, generally at a low level.

Questions for the Committee

24. Do Members have any comments on:
- a) the new data on non-neoplastic pathology?
 - b) Any other aspects of the updated information?
 - c) Taking into account the new information, the significance of the results overall?

Secretariat
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