Committee on the Carcinogenicity of Chemicals in Food, Consumer Products and the Environment

Preface

The Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC) evaluates chemicals for their human carcinogenic potential at the request of UK Government departments and agencies. The membership of the committee, agendas and minutes of the meetings, and statements are all published on the internet (http://www.advisorybodies.doh.gov.uk/coc/index.htm).

I began my tenure as chairman of the committee in April 2006. My predecessor, Professor Peter Blain, chaired the committee for nine years and I would like to express the thanks of both committee members and secretariat for his leadership and hard work during his tenure. I would also like to express our thanks to Dr Sandy Kennedy, who retired in March after many years on the committee.

During 2006, the committee has provided advice on a number of interesting and, occasionally, difficult topics. These included folic acid intake fortification and cancer risk, the possible chemical causes of testicular cancer, and a new carcinogenicity study on the artificial sweetener aspartame. We were asked by the Home Office for advice on a report which discussed animal carcinogenicity tests and, with our sister committee, the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT), provided advice on the report of the Royal Commission on Environmental Pollution on crop spraying and the health of residents and bystanders.

We are also occasionally asked to provide advice on general issues concerning developments or new ideas in carcinogenicity and in 2006 these included new publications on age-related differences in carcinogenicity and the risks of single or short-term exposure to carcinogens. We will, no doubt, continue to discuss some of these difficult topics in the future.

I have enjoyed my first year as Chairman and I thank the members and secretariat for the support they have given me during the past year. I look forward to working with them on new challenges in the future.

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Acute T25 – Possible approach to potency ranking of single exposure genotoxic carcinogens

3.1 Government departments and agencies are occasionally required to provide advice on the carcinogenic risk of a single exposure to a genotoxic carcinogen, for example, following a chemical accident. There is evidence from animal studies that a single exposure to potent genotoxic carcinogens may be associated with higher cancer risk during later life stages. At present, because the COC recommends the prudent assumption that there is no threshold for genotoxic carcinogens, it is only possible to give generic advice to the effect that any carcinogenic risk from a single exposure is likely to be very small. The committee was asked whether it might be possible to grade the potency of genotoxic carcinogens following a single exposure, perhaps by using an acute T25. It may be that any risk from a single exposure to low potency genotoxic compounds could be regarded as negligible.

3.2 A review of the available animal data on single exposure to genotoxic carcinogens and the subsequent induction of tumours indicated that there was limited information from which to assess potency and grade compounds. It was decided to run a pilot study using data on the nine most commonly studied compounds in a database of toxicological studies that assess whether a single dose of a chemical or physical agent, without extenal promotional stimuli, could cause tumour development in animals models. The rank order of potency of these nine compounds following single exposure was compared to that obtained following conventional, long-term, carcinogenicity bioassays. The premise was that, if the rankings were similar, it might be possible to make assumptions about acute potency in those many cases where data are only available from chronic studies.

3.3 The data available for this exercise proved to be limited and it was possible to calculate acute T25 values for only 5 of the 9 chemicals. Chronic T25 values were calculated for all 9 chemicals. These were compared with TD_{50} values from another database and a good correlation found. However, there was no apparent correlation in the ranking of calculated acute T25 values and chronic T25 values for the 5 chemicals for which acute T25 values could be calculated. The COC considered this to be because most of the chronic values had been obtained after oral dosing, whereas a range of routes had been used for the single dose exposures. Another potential problem was the difference in age of the animals at dosing i.e. in the acute studies the animals received a single dose, frequently when newborn, whereas in chronic studies dosing usually begins at 4-6 weeks of age and continues for most of the animals' lifetime. It was noted that it would be difficult to obtain comparative data between chronic and acute studies for the same species, strain and sex. The committee also noted that the T25 value was a concept intended to relate to long-term exposure, which could produce chronic cytotoxicity and repeated DNA repair, both of which contribute to the carcinogenic risk. Lifetime cancer incidence depends on the accumulation of risk and one would not necessarily expect a correlation between the risk from acute exposure and that from chronic exposure. Overall, the committee concluded that this approach was not useful, because of the limited data, and that there would be no value in following this further.

Age-related differences in susceptibility to carcinogenesis

3.4 The US Environmental Protection Agency (EPA) has published guidance on assessing susceptibility from early-life exposures to carcinogens. This recommends adjustment factors in the quantitative estimates of lifetime cancer risk for genotoxic chemicals to take account of different susceptibility to carcinogens at different life stages. These adjustment factors, which are 10 for exposures from birth to 2 years of age, and 3 for exposures from 2 to 15 years of age, have been developed from the limited number of studies which have compared the incidence of tumours in laboratory animals after they were dosed with a chemical at different life stages. No factors have been recommended for non-genotoxic chemicals due to insufficient information or analyses. At its July meeting, the committee reviewed two publications which developed the relevant analyses in the EPA guidance.

3.5 The papers presented a new analysis of the experimental data using statistical and modelling techniques and proposed that, for genotoxic chemicals, the sensitivity in the *in utero*, birth to weaning, and weaning to 60 day periods in test animals is 8.4, 24 and 3.7 times greater than with adult-only dosing. For the non-genotoxic chemicals, there was no increased sensitivity in the *in utero* and weaning to 60 day periods and only 3-fold higher sensitivity in the birth to weaning period. From these figures, the papers tried to draw implications for assessing human risks at different life stages. The main conclusion was that most of the total lifetime risk of cancer from continuous exposure to genotoxic carcinogens arises from exposures received before adulthood.

3.6 The committee did not agree that it would be appropriate to assume that juvenile rodents and humans would always be at a higher risk of cancer following exposure to genotoxic carcinogens than adults. It considered that this was theoretically possible for direct acting genotoxic carcinogens but not for those requiring metabolic activation. Also, in the case of non-genotoxic carcinogens, it was likely that the accumulation of mutations with age could influence the subsequent response to tumour promoting agents.

3.7 The committee concluded that there was insufficient evidence at this stage to adopt adjustment factors for genotoxic carcinogens for different life stages and did not support the conclusion that most of the lifetime risk associated with genotoxic carcinogens arose from pre-adult exposure. The topic will be kept under review.

Aspartame

3.8 The artificial sweetener aspartame was originally approved for use in 1982 and has been reviewed on a number of occasions since. In 2005, the results were published of a carcinogenicity study on aspartame carried out by the European Ramazzini Foundation of Oncology and Environmental Sciences (ERF). This study suggested that administration of aspartame was associated with an increase in lymphomas and leukaemias in rats. The committee considered the publication briefly in July 2005 and expressed a number of concerns about the design and conduct of the study. In 2006, a second, more detailed, paper of the study was published which reported, additionally, increases in preneoplastic and neoplastic lesions of the renal pelvis and ureter, malignant schwannomas of peripheral nerves and preneoplastic and neoplastic lesions of olfactory epithelium.

3.9 As part of a review by the European Food Safety Authority Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food, the FSA again sought the views of the COC on the quality of the study.

3.10 The COC concluded that there were a number of inadequacies in the conduct of the ERF study including inadequate purity assessment of the test material, the fact that the animals were allowed to reach a natural death rather than sacrificing them at the same time point, and the stated use of 70% ethyl alcohol as a fixative. The rats used in the study had a high concurrent infection rate, which may have contributed to some of the adverse findings.

3.11 As regards the interpretation of the study findings, the COC advised that it was not valid to compare the results with historical control data from a 20 year period, nor was it valid to add together all the malignant tumours in the reporting or analysis of results nor to combine the numbers of lymphomas and leukaemias. The committee concluded that the dysplasia and carcinoma of the transitional cell epithelium of the renal pelvis may have been related to the calcification which was also observed and that the findings in the renal pelvis could also be due to urinary tract infection. It commented that schwannoma is not an uncommon finding in carcinogenicity studies. As with the other reported malignancies, the slope of the dose response relationship for this finding was very shallow.

3.12 Overall, the COC concluded that, in view of the inadequacies in design of the ERF study and the use of rats with a high concurrent infection rate, no valid conclusions could be derived from it. It agreed with the evaluation of the EFSA panel that this study did not indicate a need for a review of the ADI for aspartame.

3.13 The COC statement is included at the end of this report.

"Creative Accounting": Report by People for the Ethical Treatment of Animals (PETA)

3.14 The Home Office asked the COC for advice on a report by the organisation "People for the Ethical Treatment of Animals" (PETA) entitled "Creative Accounting: (Mis)judging the costs and benefits of rodent cancer studies by the UK Home Office". The report proposed that the costs of carcinogenicity studies, in terms of animals and money, vastly exceed their benefits and that project licences should no longer be issued for these studies. It cited lack of repeatability and lack of relevance to human cancer risk e.g. a high level of false negative results. The COC's advice was sought on aspects relating to the scientific validity of carcinogenicity studies.

3.15 The COC agreed with elements of this report, for example, with the view that rodent carcinogenicity studies could over-predict the number of carcinogens, but emphasized that the studies were part of a weight-of-evidence approach to carcinogen risk assessment. Carcinogenicity studies were not used in the simplistic manner implied by the report. They were peer-reviewed and all data used in the overall evaluation. The committee did not agree with the assertion that approximately two-thirds of chemicals listed by the International Agency for Research on Cancer (IARC) as known human carcinogens showed no carcinogenic effects when tested in rodents, nor with the implication that there was no value in the IARC classifications of "not classifiable" or "possibly carcinogenic". Most of the agents classified by IARC as Group 1 and 2A human carcinogens were positive in animal studies.

3.16 The committee also commented that it was important to understand the genesis of NTP studies and noted that they were not designed for the purposes of quantitative risk assessment. High dose levels were used in long term rodent carcinogenicity studies to keep the numbers of animals required to detect a 5-10% increase in tumour incidence to a minimum of 50 animals/dose group.

3.17 The COC concluded that it was likely that long-term rodent studies would be needed for the identification of potential human carcinogens for the foreseeable future. However, it was important that all studies are conducted to internationally agreed guidelines.

Folic acid fortification and carcinogenesis

3.18 Folate is the generic term for a naturally occurring family of B-group vitamins comprising an aromatic pteridine ring linked to p-aminobenzoic acid and a glutamate residue. Folic acid (pteroylmonoglutamic acid) is the synthetic form commonly used in supplements and food fortification. In 2000, the Committee on the Medical Aspects on Food and Nutrition Policy (COMA) concluded that the universal fortification of flour with folic acid would significantly reduce the number of conceptions and births affected by neural tube defects (NTDs). In 2003, the Scientific Advisory Committee on Nutrition (SACN) was asked to assess the evidence that had arisen since the COMA publication. In its draft report on 'Folate and Disease Prevention', published in 2005, the SACN recommended that mandatory fortification of flour should be introduced to reduce NTD risk but, subsequently, it requested further time to reconsider its advice following some concerns about high intakes of folic acid and cancer risk.

3.19 SACN asked the COC to review the relevant data and to give its opinion on whether dietary folic acid intake is associated with increased cancer risk. The COC noted that animal data suggested that timing of the folic acid administration could be an important factor in potential cancer risk since the data showed that high doses of folic acid may progress the development of pre-existing neoplasms. The data suggested a possible effect on inherited accelerated colorectal tumourigenesis in mice but not on sporadic colorectal tumourigenesis, although the comment was made that the animal data were equivocal and should not be over-interpreted. Overall, the Committee agreed there were multiple plausible mechanisms, including epigenetic mechanisms, whereby folic acid may influence cancer risk.

3.20 Most epidemiological studies indicate a reduced risk of cancer with increased folic acid or folate intake. The COC noted that, in many of the studies, the folic acid had been taken in multivitamins, and the presence of other micronutrients in the multivitamins was a complicating factor. However, the committee agreed that preliminary results from one unpublished, randomised trial showed a significant increase in adenoma multiplicity in subjects with a recent history of colorectal adenomas who had been supplemented with folic acid (1 mg/day) for over 3 years. It also noted the results of the two studies of folic acid supplementation on cardiovascular disease outcomes which showed non-significant associations between folic acid (in combination with either vitamin B12 or B12 and B6) and cancer. The committee noted that there may be susceptible subgroups in the population. It was not possible at this stage to identify these. Factors which might be relevant were age and the presence of pre-neoplastic conditions.

3.21 A review by the COM of background variation in micronuclei in peripheral blood lymphocytes had indicated that there was good evidence from cross sectional and intervention studies to suggest that plasma or serum folate levels were negatively correlated with micronucleus formation. It was noted that whether or not risk is increased or reduced may depend on a balance between the thresholds for epigenetic promotional effects and reduction of DNA damage.

3.22 The committee agreed that it remains unclear whether the possible deleterious effects of high folic acid outweigh the known and potential health benefits and that this balance may differ across individuals and populations by genetic characteristics and by life stage. It was noted that, in the US, where mandatory folic acid fortification was introduced in 1998, voluntary fortification of other foods continued to be permitted and blood analyte data indicated that current intakes of folic acid were higher than planned. In conclusion, a precautionary approach was recommended in considering mandatory fortification of flour with folic acid.

Royal Commission on Environmental Pollution report on crop spraying and the health of residents and bystanders

3.23 In August 2004, the Royal Commission on Environmental Pollution (RCEP) announced a review of the scientific evidence on which the Department of the Environment, Food and Rural Affairs (Defra) had based its decision on bystander exposure to pesticides (i.e. exposure of members of the public who may be in the vicinity of an area sprayed with pesticide) and its policy on access to information on crop spraying. The RCEP published its report in September 2005. The COC and COT were subsequently asked by Defra and the Advisory Committee on Pesticides (ACP) to comment on RCEP report. The COC discussed the report at its March 2006 meeting and focussed on those sections relevant to its area of expertise.

3.24 The COC noted that the RCEP had not had time to undertake a rigorous evaluation of all the available epidemiological literature and had not distinguished clearly between hypothesis generating studies, and analytical studies which could be used to define dose-response relationships for pesticides associated with cancer and were of importance in the assessment of causality. The RCEP had recommended a comprehensive systematic review of the literature in this field which would take account of, and avoid, the shortcomings of the 2004 report on pesticides of the Ontario College of Family Physicians. This report had been considered by the COC epidemiologists in 2004 and the committee recalled that the main problems identified were the selection of data (which did not include available negative data), the selective interpretation of results, and the lack of good exposure data in most studies. This last problem could not be remedied by a future review.

3.25 Members agreed with the RCEP that better exposure measurement in cancer epidemiology studies was a high priority for further research. The COC agreed that appropriate biomonitoring studies (eg using biomarkers of exposure or of biological effect) would be helpful in any population studies of cancer but noted that exposure at, or following, cancer diagnosis might have little relevance to that which could have been causal. It was not considered that there was a need at present for more encouragement for the development of newer animal models for cancer, particularly in the area of cancer hazard identification.

3.26 A joint COT/COC statement is included at the end of the COT section of this report.

Testicular cancer

3.27 The COC had decided to review the possible chemical aetiology of testicular cancer at the 2005 horizon scanning discussion. At its March 2006 meeting, the committee reviewed a paper which gave an overview of background information and risk factors in testicular cancer.

3.28 Testicular cancer is a relatively rare cancer worldwide, accounting for approximately 1-2% of all male cancers diagnosed. However, it is the commonest cancer in men under 45 years old (accounting for 17% of all cancers occurring in this group). The incidence of testicular cancer varies widely around the world and varies with ethnicity. Gradual increases in incidence have occurred in many countries since the 1960s, and the increase is unexplained. Survival rates have improved in recent decades, possibly as a result of earlier diagnosis, and survival of testicular cancer is the highest of any cancer in men in the UK.

3.29 Well-established indicators of risk for testicular cancer are cryptorchidism and carcinoma *in situ* but there is currently no agreement about the involvement of other factors in its aetiology. Credible hypotheses that have been proposed involve *in utero* risk factors, maternal hormonal patterns and dietary practices.

3.30 Several reports have suggested that men engaged in some occupations may be at higher risk for testicular cancer. These include men working in white collar occupations, aircraft workers, leather workers, paper and printing workers, firefighters and men working in agriculture. However, there are conflicting views in the literature. The committee noted that men in white-collar jobs were likely to be of higher socio-economic class that those in blue-collar jobs.

3.31 There are currently no published epidemiological studies that examine an association between exposure to endocrine disrupting chemicals and testicular cancer. A recent analysis found no association between the antiandrogen dichlorodiphenyldichloroethylene (DDE) and testicular cancer some 2-22 years later. It was noted that, at a recent COT seminar on adverse male reproductive effects, the predominant view was that the problem arose *in utero* and that, if the increase in testicular cancer was part of the testicular dysgenesis syndrome, a wider approach would be needed to understanding the cause(s). There was evidence of genetic predisposition to testicular cancer, with the gene responsible being carried on the X chromosome, but this did not explain why the incidence had increased.

3.32 In a number of papers an increased intake of milk and dairy products has been associated with increased testicular cancer risk and it was hypothesised that may be associated with exposure to oestrogen in the milk. However, the committee was informed that UK consumption of all cows' milk and cream has fallen by 33 per cent between 1975 and 2002-03 and that the oestrogen content of milk has not changed since the 1970s. It was further noted that the bioavailability of oestrogens from cows' milk would be low (the bioavailability of oestradiol is less than 5%).

3.33 Overall, no clear chemical aetiology was identified and the committee decided not to pursue this issue further.

"Tissue Organisation Field Theory" of carcinogenesis

3.34 A recent paper had claimed that it is feasible that chemical environmental contaminants could be major factors in cancer aetiology. The committee considered this paper and commented that it was neither a systematic nor reasoned review of the literature. However, the paper had referred to the "tissue organisation field theory of carcinogenesis (TOFT)" and the committee was asked to consider this hypothesis at its July 2006 meeting. In summary, TOFT assumes that proliferation is the default state of cells and that sporadic cancers arise when pathogens or carcinogens disrupt the biological interactions between different cell layers. The mutations present in neoplastic cells are considered to be incidental, not causal.

3.35 The COC considered that this hypothesis contained some interesting ideas but commented that the *N*-methyl-*N*-nitrosourea (MNU) model which had been used to give support to the hypothesis was odd, in that there was evidence that the *ras* mutation was not caused by MNU but was present in cells already and that MNU propagated clonal expansion of these cells. It was noted that cell cycle activity requires not just mitogenic stimuli but also survival signals, only a proportion of cells in any cancer are truly stem cells that can contribute to a new colony of cells, and that tumour suppressor gene inactivation is necessary but not sufficient to cause cancer. The committee also noted the increasing literature on the importance of epithelial-mesenchymal interactions in causing and sustaining tumours and of epigenetic changes that may precede or accompany mutation.

3.36 Overall, the committee considered that the hypothesis contained some interesting ideas but that there were insufficient data to support it.

Horizon scanning

3.37 The COC undertakes "horizon scanning" exercises at regular intervals to identify new and emerging issues which have the potential to impact on public health. After an extensive literature search, a number of topics were identified by the secretariat for consideration by the committee at the 2006 exercise. From these and committee members' own proposals, the COC considered that the following topics should be taken forward:

- formaldehyde carcinogenicity
- oxidative damage to DNA as a carcinogenic mechanism of action
- the role of toxicogenomics in predicting carcinogenicity and in confirming or proposing a mechanism of action (joint review with the COT and COM)
- the characterisation of the dose-response at low exposures to genotoxic carcinogens
- recent developments on the Human Relevance Framework (HRF)
- the carcinogenicity of depleted uranium, including uranium pyrolysis.

3.38 The committee also decided that further work on the carcinogenicity of mixtures and on mutational fingerprints should be taken forward jointly with the COM, and that developments in the assessment of the carcinogenicity of nanoparticles should be kept under review.

Ongoing topics

Comparative risk assessment

3.39 The COC and COM are currently discussing the ways in which the carcinogenic risk of chemicals might be better communicated and put into context alongside other risks.

HSE Disease Reduction Programme: project on chemical carcinogens

3.40 At its March meeting, the Health and Safety Executive (HSE) provided the committee with a brief update progress in a high priority initiative to identify the scale and reduce the incidence of occupational cancer, and invited COC members to work with the HSE them to influence the process. Further updates will be made as the project progresses.

Betel quid, Pan Masala and areca nut chewing

3.41 At its November meeting, the committee discussed new data on the carcinogenicity of areca nut, an ingredient of betel quid or pan masala, which is chewed as an aid to digestion and as a stimulant. A statement will be published in 2007.

Prostate cancer and pesticide exposure

3.42 At the November meeting, the committee was also asked by the Pesticides Safety Directorate of Defra for its views on a report commissioned from the Institute of Occupational Medicine entitled "Desk study on prostate cancer and pesticide exposure" and on whether the report alters the conclusion in the COC's 2004 statement on prostate cancer. An additional statement will be published in 2007.

Statements of the COC

Statement on the Carcinogenicity Study of Aspartame by the European Ramazzini Foundation

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	2,000	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	2,000	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	2,000	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	2,000	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.

COC discussion

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 witha 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.

Statement COC/06/S2

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Mr S Robjohns BSc MSc	Scientific – Health Protection Agency

Declaration of interests during the period of this report

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Professor D H Phillips (Chair)	Aviva Banco Santander BG Group Bradford & Bingley Centrica CGNU Lattice Group National Grid	Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder	NONE	NONE
Professor P G Blain (Chair until 31.3.06)	NONE	NONE	NONE	NONE
Dr C Allen	NONE	NONE	NONE	NONE
Prof A Boobis OBE	Bank Santander Barclays Bank BG Group BT Group Centrica Halifax National Grid Transco Scottish Power Astellas Pharma	Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Consultancy	GlaxoSmithKline ILSI HESI	Research Support Unpaid member of Board of Trustees
Dr P Carthew	Unilever	Salary	NONE	NONE
Prof P B Farmer	Santander Bradford & Bingley Foreign & Colonial Friends Provident Health Effects Institute Torotrak	Shareholder Shareholder Shareholder Shareholder Research Committee Member Shareholder	American Chemistry Council CEFIC	Research Support Research Support
Mrs R Glazebrook	BT Group Lloyds TSB National Grid	Shareholder Shareholder Shareholder	NONE	NONE
Prof D Harrison	The Forensic Institute Crusade Laboratories	Shareholder Consultancy (dormant)	NONE	NONE
Ms D Howel	NONE	NONE	NONE	NONE

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Dr S J Kennedy (Member until 31.3.06)	Unilever	Shareholder	NONE	NONE
Dr B G Miller	Scottish Power	Shareholder	NONE	NONE
Prof R A Roberts	AstraZeneca HBOS P & O	Salary Shareholder Shareholder	NONE	NONE
Prof D E G Shuker	NONE	NONE	NONE	NONE
Dr P Vineis	NONE	NONE	NONE	NONE
Dr N Wallis	Pfizer	Salary Shareholder	NONE	NONE