REPORT OF THE MEETINGS ON ASPARTAME WITH NATIONAL EXPERTS

QUESTION NUMBER: EFSA-Q-2009-00488

The following experts were nominated by the Advisory Forum members to take part in the meetings on aspartame: Stephen Atkin*, Maria de Lourdes Bastos, Jacqueline J. M. Castenmiller, Rhodri Evans, Tor Øystein Fotland, Tero Hirovonen, Trine Husøy, Nils-Gunnar Ilbäck*, Indre Karpoviene, Eleni Kakouri, Erik Konings*, Maria Conçeição Branco Montenegro*, Angelina Pena*, André Penninks*, Aldert Piersma*, Iona Pratt, Hannu Raunio, Natalie Thatcher*, Marica Theiszová*, Martina Valachovičová, Daniela Winklerová

(* indicates expert was part of the Organising Team)

The conclusions and recommendations of this report reflect those of the national experts involved in the meetings on aspartame and do not necessarily represent the views of EFSA.

ON-1641 Noted at the 36th Advisory Forum Meeting, 19-20 May, 2010

SUMMARY

The Advisory Forum of EFSA discussed the issue of aspartame in 2007 and at the 24th AF meeting held on 6-7 December 2007 it was agreed that a special meeting would be held on aspartame. This meeting of National Experts was to take place early in 2009 and, as provided in the Terms of Reference endorsed by the Advisory Forum, was to review the information available on aspartame; agree on the completeness of the information ensuring any missing data was added; identify possible data gaps and discrepancies in the available data and where such exist to consider detailed options to address the outstanding issues.

An Organising Team, nominated by members of the Advisory Forum, was tasked with preparing for the meetings of National Experts by identifying, collating and reviewing all published papers on aspartame since the review carried out by the Scientific Committee on Food in 2002. In addition the Organising Team also considered available non-peer reviewed information and anecdotal claims by individuals who attributed various symptoms and illnesses directly to aspartame consumption. A meeting was held with interested parties who had submitted information in response to a 'call for data' published by EFSA in September, 2008.

Each of the areas considered, including exposure data, brain function, satiation and appetite, allergenicity and immunotoxicity, metabolic aspects and diabetes, carcinogenicity (including cancer epidemiology) and genotoxicity were considered by the Organising Team and reported on. For each, consistent with the Terms of Reference for the National Expert Meeting, they made preliminary conclusions on whether there were data gaps or discrepancies which required further consideration. This report represents the results of these deliberations.

Overall, the Organising Team did not identify any major gaps in information on aspartame and the National Experts agreed with their view. However, several suggestions are made within this report for additional data which would add to the available knowledge on aspartame and its metabolites. To address the communications element of the Terms of Reference a workshop with stakeholders and other interested parties is to be arranged to share and discuss the findings of the work.

In conclusion, the National Experts have not identified any new evidence that requires a recommendation to EFSA that the previous Opinions of EFSA and the SCF need to be reconsidered.

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BACKGROUND

Aspartame (L-aspartyl- L-phenylalanine methyl ester) is a low-calorie, intense sweetener. It is a white, odourless powder, approximately 200 times sweeter than sugar. Aspartame has the chemical structure as described in Figure 1. It is used in a number of foodstuffs such as drinks, desserts, sweets, dairy, chewing gums, and weight control products and as a table-top sweetener throughout the world.

The approval and safety of aspartame

Aspartame was originally proposed for approval in the US for dry foods in 1974. Following lengthy investigations and hearings into the operations of G. D. Searle and the validity of the studies supporting the safety of aspartame, a Public Board of Inquiry was convened in 1980 which concluded that aspartame should not be approved pending further studies. In 1981 the FDA approved the use of aspartame for dry products on the basis of evidence from additional studies. In 1983 it was further approved for use in carbonated drinks and subsequently for use in all foods. The sweetener has now been authorised worldwide.
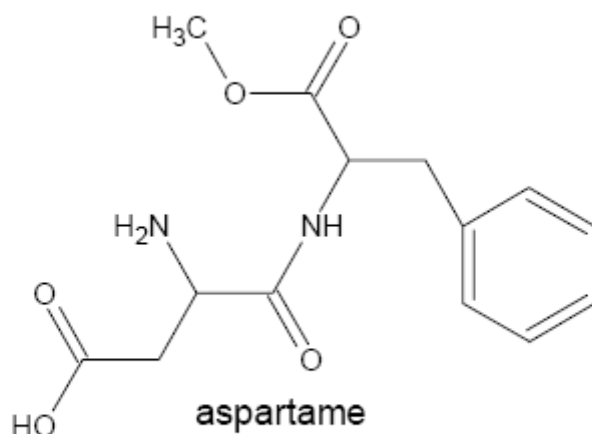

Figure 1: Chemical Structure of Aspartame

The sweetener and its metabolic breakdown products (phenylalanine, aspartic acid and methanol) have been a matter of extensive investigation for more than 20 years including experimental animal studies, clinical research, intake and epidemiological studies and post-marketing surveillance. After oral ingestion, aspartame is hydrolysed, either within the lumen of the gastro-intestinal (GI) tract, or within the mucosal cells lining the inside of the GI-tract. Hydrolysis is be facilitated by specialised enzymes in the intestines (esterases, peptidases), rather than by the acidic conditions in the stomach. The products that result from these reactions are methanol and the amino acids aspartic acid and phenylalanine. At doses relevant to human consumption, hydrolysis is very efficient; the amount of aspartame that enters the bloodstream is not detectable (SCF 2002). The safety issues that have been raised in the past about aspartame have included: (1) the possibility of toxicity from methanol and/or its systemic metabolite formaldehyde; (2) elevations in plasma concentrations of phenylalanine and aspartic acid, which could result in increased transport of these amino acids into the brain, altering the brain's neurochemical composition; (3) the possibility of neuroendocrine changes, particularly increased concentrations of catecholamines derived from phenylalanine and its hydroxylation product,

tyrosine in the brain, synaptic ganglia and adrenal medulla; and (4) a postulated link with epilepsy and brain tumours (SCF, 2002). The ADI of 40 mg aspartame/kg bw established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) was reaffirmed in the review and opinions of the Scientific Committee on Food (SCF) in 1985, 1989, 1997 and 2002.

Reviews of safety and on-going public concerns about aspartame

Subsequent scientific reviews and opinions since 2002 (such as the EFSA 2006 Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to a new carcinogenicity study on aspartame, Question Number EFSA-Q-2005-122) did not lead to concerns suggesting the need for re-appraisal by Regulatory Authorities with respect to the conclusions of the SCF. However there continues to be public concern with respect to the health risks of aspartame use in food.

These concerns were first raised in the United States and subsequently noted in EU Member States in relation to procedural aspects associated with the approval process for aspartame and they continue to be expressed. Numerous anecdotal reports of adverse reactions following dietary exposure to aspartame have also been documented.

The response of EFSA and EU Member States to public concerns

EFSA, together with its Advisory Forum (AF) which is made up of representatives of the risk assessment bodies in the EU Member States, agreed to look into the basis for this public concern. In order to do this, arrangements were made to hold a meeting of national experts with relevant scientific knowledge, nominated by the Member States. The first meeting was held on 2-3 April, 2009 and a further meeting took place in November 2009. A third meeting was held in January 2010 to finalise the report.

The aim of this initiative, facilitated by EFSA as requested by its AF and bringing together experts nominated by Member States, was to address the on-going public concerns.

In considering the approach to take, it was agreed that the preparatory work would be carried out by an Organising Team, rather than establishing a Panel Working Group. This maintains the option of EFSA consulting the Food Additives and Nutrient Sources added to food (ANS) Panel regarding the outcome of the meeting of national experts.

Tasks of the Organising Team

At the 24th AF meeting held on 6-7 December 2007, the issue of aspartame was discussed and it was agreed that a national experts meeting would be held on aspartame. The AF members were asked to nominate representatives to form an Organising Team to prepare for a National Experts meeting. The mandated tasks of the group were:
- to conduct a thorough analysis of studies and other information available on aspartame
- specifically to identify new scientific evidence available since the last evaluation by the SCF in 2002
- to identify non-peer reviewed information available
- to consider possible limitations in current knowledge.

The preparatory work of the Organising Team has informed and been used as a basis for the discussions at the National Experts meetings on aspartame. It was not within the remit of the Organising Team or the National Experts to consider non-scientific aspects of the approval, marketing and use of aspartame.

TERMS OF REFERENCE

The Terms of Reference for the meetings of national experts were drafted in April 2008 and following revision were agreed by the AF in September 2008.

The text of the Terms of Reference is as follows:

An EFSA Meeting of National Experts on Aspartame will be convened tentatively in December 2008 to identify possible areas of discrepancies, or gaps, in the body of evidence on aspartame safety and to consider options to address these discrepancies and/or gaps, if any. Specifically, the meeting of National Experts on Aspartame will:

- Review a comprehensive overview of reports, publications and other data on aspartame, provided by EFSA;
- Agree on the completeness of the overview provided by EFSA or, alternatively, ensure that missing data are added;
- Identify possible data gaps and discrepancies in the available data;
- In case no data gaps or discrepancies are identified, agree on a (communication) approach to confirm the adequate robustness of the data, supporting the absence of any human health concern;
- In case such data gaps or discrepancies exist, consider detailed options to address these outstanding issues and agree on a preferred approach for work, if needed;
- Reach agreement on the organisation of the work, including parties involved and the timing of the work, taking into account: resource implications and the need for independent scientific evaluation of the outcome of the work.

1. Introduction

In order to prepare for the meetings of the National Experts, the Organising Team held ten meetings between April 2008 and October 2009.

The steps taken were as follows:

- Establishment of a database of scientific literature
- Allocation of specific areas and endpoints[1] to members of the Organising Team
- Combination of specific endpoints reports
- Call for data from stakeholders
- Collation and evaluation of information from stakeholders
- Establishment of a database of anecdotal case information
- Preliminary drafting of this background report

2. Objectives

The objectives of the Organising Team were to identify, collate and review all published papers since the review carried out by the SCF in 2002. In addition the Organising Team considered available non-peer reviewed information and anecdotal evidence. As part of the review, the Team considered and identified data gaps in the available knowledge identifying areas where further work may be considered necessary and on this basis made conclusions and recommendations for consideration at the national expert meetings.

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[1] Biological area chosen for review for possible effects

METHODOLOGY

1. Meetings

The Organising Team held ten meetings in addition to an open meeting with stakeholders and meetings of National Experts as follows:

Meeting Date	Location	Activity
17-18 April 2008	Parma	Agree Mandate/TOR and approach to be taken
14 July 2008	Parma	Preliminary findings on Genotoxicity, Carcinogenicity, Exposure and Epidemiology. Identification of subsequent areas – Brain Function, Metabolism, Reproduction/Fertility, Immunotoxicity/Allergenicity, Satiation/Appetite
16 September 2008	Brussels	Findings and Conclusions of End Points, Discussion on 'Claimed Effects'
26-27-28 November 2008	Parma	26 – Hearing with interested parties who made submissions 27-28 Discussion on submissions and the draft report.
28-30 January 2009	Parma	Further discussion on submissions and the draft report.
11-13 March 2009	Parma	Preparation for National Experts meeting, April, 2009.
2-3 April, 2009	London, UK	First meeting of the National Experts
6-7 May, 2009	Parma	Discussion on anecdotal case details, database development and revision to draft report
15 June, 2009	Parma	Discussion on database development and draft report
14-15 September, 2009	Parma	Detailed discussion on data analysis
22-23 October, 2009	Parma	Discussion of full draft background report including new/updated sections and preparation of the national expert meeting of 10-11 November
10-11 November, 2009	Porto	Second National Experts meeting to discuss updated background report including new section on anecdotal data
19-20 January, 2010	Parma	Third National Experts meeting to conclude on the information prepared by the Organising Team

2. Information Review

2.1 Scientific Literature

It was agreed at the first meeting of the Organising Team in April, 2008 that the existing opinions of JECFA (1980) and the former SCF (1985, 1989, 2002) would be considered valid, sufficiently comprehensive, and covering all relevant literature until 2002. Hence these opinions were considered as sufficient key references for the literature prior to the latest SCF opinion, expressed on 4 December 2002 and all literature considered therein would not be reconsidered by the Organising Team.

Consequently, the focus was to be on collecting and analysing any new publication after 2002 with a view to conclude on:

- whether the previous JECFA and SCF opinions still hold in the view of new knowledge;
- whether all endpoints/effects as reported in humans are adequately addressed, or whether gaps are identified;
- whether the available literature on these endpoints/effects express conflicting views; and
- whether recent studies reveal effects on endpoints not considered previously.

It was agreed that in order to undertake the literature review, EFSA would coordinate the search and establish a data set of papers available, which was shared with the Organising Team experts.

The literature database included scientific peer reviewed papers and relevant non-peer reviewed papers (such as technical reports and published conference proceedings).

As of 30 January, 2009 the database contained a total of 1092 references relating to aspartame. This included pre-2002 publications.

2.2 Case Reports

In addition to the peer reviewed scientific papers, the Organising Team considered case reports published in peer reviewed journals and also other reports compiled by Dr H.J. Roberts and published under the title 'Aspartame Disease – An ignored Epidemic' (Roberts, 2001).

3. Areas Considered

Each of the experts involved in the Organising Team was allocated one (or more) of the areas identified below.

- Exposure Data
- Brain function
- Effects on satiation and appetite
- Allergenicity and Immunotoxicity
- Metabolism of endogenous and exogenous compounds
- Carcinogenicity (including cancer epidemiology) and genotoxicity

For each 'endpoint' all the literature available in the database was reviewed and the four aspects listed as bullet points in Section 2 above were considered. While the Organising Team originally intended to report on the areas of 'Epidemiology, including exposure' and 'Carcinogenicity' separately, it concluded that Epidemiology and Carcinogenicity should be reported together and Exposure reported separately. Similarly, for 'Reproduction/Fertility' the Organising Team identified no new studies in

this area and did not consider the endpoint to be of specific concern in relation to aspartame. The section was, therefore, omitted.

In addition the Organising Team considered the available, but non peer reviewed, 'case histories' which largely consisted of anecdotal claims by individuals who attributed various symptoms and illnesses, including headaches, seizures, memory loss, vision/eye conditions, allergies and gastro-intestinal symptoms directly to aspartame consumption.

The Organising Team experts considered all relevant publications identified, produced a summary table of the publications and provided comment on the strength of the evidence from the study, noting any limitations. These summary tables are included in each section.

4. Call for Data

In order to ensure that all stakeholders were given the opportunity to submit relevant information for the Organising Team to consider, a public 'Call for data' was published on the EFSA website on 25 September 2008 and subsequently sent directly to stakeholders via the EFSA Stakeholder Consultative Platform. The call for data closed on 31 October 2008.

All the stakeholders submitting information to the call and indicating an interest in follow up were provided with the opportunity to supplement their submission with a personal presentation. A hearing with the Organising Team was arranged for the 26 November 2008. A short presentation was made by each stakeholder followed by the opportunity for exchange of questions. The stakeholders who took part represented different interest groups and included aspartame public interest groups, trade organisations, a researcher and an individual who believed aspartame consumption was responsible for a range of health problems suffered.

In all 26 submissions were received. Of these 13 were from private individuals, 7 of which provided details of a range of illnesses and symptoms claimed to be associated with aspartame use.

Of the remainder, 6 were from researchers or research institutions with an interest in the subject, 3 were corporate submissions from food businesses producing sweeteners and 4 were from special interest groups with a view on aspartame use.

All the submissions were reviewed to ascertain whether the respondent had identified any research which had not previously been considered by the Organising Team, or any additional information which identified gaps in the current knowledge, or the need for further considerations.

All of the information in the submissions was subsequently considered amongst the non-peer reviewed data or added to the peer reviewed literature database for consideration. Where the submission related to a specific end point, the expert associated with that particular end point reviewed the information. Submissions that related to papers already addressed previously by the SCF were not considered further.

In addition to identifying papers for consideration, anecdotal information on cases of reported effects were also submitted by interest groups. This information was used along with the anecdotal data and is dealt with under the section 'Review of Anecdotal Data'.

Many of the submissions, in particular those from interest groups, raised matters outside the remit of the Organising Team, for example concerns were raised regarding the way that aspartame was originally approved in the US, the robustness of the research submitted at the time of the application for approval and the operation of the SCF in 2002 was also questioned. These submissions were also

considered to determine whether they raised issues relating to the scientific basis for determining the safety of aspartame. Where the details related to non-scientific aspects of aspartame use (such as marketing and legislative controls), the submissions were not considered further as it was not within the remit of the Organising Team to do so. It is acknowledged, however, that these submissions are indicative of the ongoing public concerns regarding the safety of aspartame.

Details of all documents reviewed which were not included in the literature search are summarised in Appendix 1

AREAS REVIEWED

1. Exposure Data

In 2002 the SCF concluded that the estimates of intake by mean and high-level consumers were fairly consistent between European countries (UK, Finland, The Netherlands, Norway and France) based on the available data. High-level consumers, both adults and children, were unlikely to exceed the ADI of 40 mg/kg bw per day for aspartame, including sub-groups such as diabetics who are likely to be high consumers of foods containing aspartame.

Eleven studies published since 2002 estimating aspartame intake from foods have been identified. Seven were published in peer reviewed journals, while four reports have been published by Food Safety Authorities; The Food Standards Agency (FSA) (UK), Food Standards Australia and New Zealand (FSANZ) (Australia, New Zealand), Voedsel en Waren Autoriteit (VWA) (The Netherlands) and the Vitenskapskomiteen for mattrygghet (VKM) (Norwegian Scientific Committee for Food Safety). The reported studies have been reviewed extensively by Renwick (2006) and Magnuson et al. (2007). The summary below shows the key results from these and other published studies.

1.1 Summary Table: Exposure Data

Study	Country	Age range (years)	Study Population	Study features	Aspartame intake Mean mg/kg bw per day (Percentile)
Arcella et al. 2004	Italy	14-17	Aspartame consumers + non consumers	Intake based on 12-d (3x4) dietary record and levels manufacturers	0.04 (95%ile 0.2)
Chung et al. 2005	Korea	>1y	Aspartame consumers + non consumers	1-day 24h recall, National Nutrition Survey 1998 reference values (1997 study) for candy, ice cream, yoghurt, soft drinks and So-ju (traditional liquor)	0.14 (95%ile 6.4)
Devitt et al. 2004	Canada	2-6	Type 1 Diabetics	1-day 24h recall. Data obtained from product labels and direct communication with manufacturers	4.1 (90%ile 7.8)
FSA 2003	UK	1.5-4.5	Aspartame consumers	7-day Diary survey soft drinks, dilutable drinks, levels reported by manufacturers (dilutable drinks analysed)	3.38 (97.5%ile 12.01)

FSANZ 2004	Australia and New Zealand	>12	General population (high potential intakes) and diabetics/impaired glucose tolerance	Prospective 7-day food diary focused on key products including brands that would contain intense sweeteners. Data supplied by manufacturers	2.4 (95%ile 7.0) 2.3 (95%ile 7.5)
Illback et al. 2003	Sweden	0-90	Type 1 and Type 2 Diabetics (aspartame consumers)	Intake based on MPL*, intake retrospective FFQ#	<6
Leth et al. 2007	Denmark	1-80	Aspartame consumers + non consumers	7-day dietary record, soft drinks, analytical data	0.1 (90%ile 0.7) high intake 0.8 (90%ile 4.0; 99%ile 9.4)
Lino et al. 2008	Portugal	Teenage students (n=65)	Aspartame consumers + non consumers	FFQ for soft drinks and light nectars, 2007. Aspartame concentrations based on analytical data.	1.1
Magnuson et al. 2007	USA	1-70+	Aspartame consumers	NHANES food consumption survey 2001-2002. Number of sources used for aspartame concentration in foods.	4.85 (95%ile 13.3)
Norwegian Scientific Committee for Food Safety 2007	Norway	1-adults 1-79	Aspartame consumers + non consumers	Norwegian dietary surveys 1997-2000. Exposure calculated from soft drinks, 'saft' and nectar based on actual content level and sales volumes reported by industry. High intake scenario if 100% consumed drinks contain aspartame	highest intake 1-2 y children: 2.3 (95%ile 8.4) Upper level: highest in 2 and 13y: 3.6 (95%ile 10.2)
van Rooij-van den Bos et al. 2004	The Netherlands	1-97	Aspartame consumers + non consumers	Dutch National Food Consumption Survey 1998, 2-day dietary record, analytical data aspartame in soft drinks, dilutable drink, yoghurt drink, fruit juice, yoghurt, vitamin supplements High Intake scenario: highest consumption	0.1 (95%ile 0.5) Upper Level 1-4 y: 8

				(95%ile) of each category (users) assuming all products in each category consumed were sweetened using average measured concentration with single sweetener	

*MPL: Maximum permitted Level

#FFQ: Food Frequency Questionnaire

Discussion

In general, the average intake of aspartame is below 5 mg/kg bw per day with a highest intake reported of 13 mg/kg bw per day. This has been estimated in several ways, including high intake scenarios assuming high intake including beverage content to be at maximum permitted level or analytical measurement of aspartame content or based on information from manufacturers own composition data. All values reported here were well below the ADI. The studies have their strengths and weaknesses, including current relevance of food consumption survey, food sources of aspartame included, representative product concentration data, time base for intake calculation, study population and high intake scenarios as discussed by Renwick (2006) and Magnuson et al. (2007), and described more extensively below.

In addition to the studies mentioned, estimates of aspartame intake were given in a European Commission report on dietary food additive intake (2004), using the "Tier 2" approach (Tier 2 refers to the assessment at the level of individual Member States, combining national data on food consumption with the maximum permitted usage levels for the additive). In this report a maximum intake of 40% of ADI was calculated for the Netherlands and UK.

Current relevance of food consumption survey

Most of the consumption surveys which are used for aspartame intake calculation originate from before 2000 and it should be noted that consumption patterns may have changed in the intervening years. Studies by FSA (2001), Arcella et al. (2001), Magnuson et al. (2001-2002), FSANZ (2002-2003) and Lino et al. (2008) were carried out since 2000.

In the US the use of carbonated diet soft drinks has increased from 2002 to 2004 from about 53 litres per year per capita to 60 litres per year per capita (Magnuson et al. 2007). This indicates that the consumption of aspartame is likely to have increased since 2002. Data from the Netherlands showed an increase in the annual consumption of soft drinks containing sweeteners from 12 litres per capita in 1995 to 30 litres per capita in 2006.

Food sources of aspartame

Most of the intake estimates are based on the use of soft drinks alone as this appears to be the major source of exposure to aspartame. Food analysis of products in the Netherlands showed the presence of aspartame in dilutable drinks, fruit lemonade, yoghurt drinks, yoghurt and vitamin supplements. In general, aspartame concentrations were one tenth of the maximum permitted levels with the exception of vitamin supplements, which contained a mean level of 6.4 mg/g, exceeding the maximum permitted level of 5.5 mg/g (van Rooij-van den Bos et al. 2004). Also tabletop sweeteners are a category which may not be included in all intake calculations.

Product concentration data

In general aspartame concentration data in foods are not based on analytically measured values in foods. Only the studies of Leth et al. (2007), van Rooij-van den Bos et al. (2004), and Lino et al. (2008) are based on analytical data. From these data it can be concluded that on average products contain one tenth of the maximum permitted levels of aspartame with the exception of soft drinks which contain approximately 25% of the maximum permitted level.

Other concentration data used for the exposure estimate are based on maximum permitted levels (1 study) and data from product manufacturers (6 studies). Details of specific products or brands are not given and thus it is not clear what levels were used and whether all relevant information was complete. In the study of Chung et al. (2005) it was unclear what composition data were used.

Intake calculation

In most of the studies the estimated aspartame intake is based on the multiplication of amounts of each food product consumed and concentrations of aspartame present. The results of all products are summed and divided by the body weight. For comparison with the ADI as part of risk characterisation the average long-term intake by an individual would be needed. Therefore intake calculations should preferably be based on probabilistic modeling such as Monte Carlo (Leclercq et al. 2003). Intake data for one single day underestimates the proportion of the population who will be consumers of this food additive, but can grossly overestimate the average intake in those individuals.

Study population

It is preferable to include high-risk groups in intake calculations such as diabetics (both type 1 and type 2), children, pregnant women and those who are on weight control programmes. Most studies (10) report intakes for the average population, only 3 studies reported intake information including individuals with diabetes. The larger studies also reported on aspartame intakes for specific age groups.

High intake scenarios

High intake scenarios performed by Arcella et al. (2004), Illback et al. (2003), the Norwegian Scientific Committee for Food Safety (VKM) Report (2007), and van Rooij-van den Bos et al. (2004) suggested that chronic intakes will not reach the ADI.

High intake scenarios are generally based on the assumption that aspartame will be the source in all sweetened food products at the highest permitted concentrations. The authors conclude that only a small percentage of the population would exceed the ADI (Ilback et al. 2003: children).

According to Magnuson et al. (2007), reported exposures are not representative of levels currently used in foods and beverages and will not be representative in the future for the following reasons:

- Manufacturers will use blends of sweeteners

- Aspartame is one of the more expensive sweeteners

- New sweeteners will be placed on the market such as sucralose which will possibly replace aspartame

- There is no incentive for producers to add more sweetener than is needed

The conclusions and recommendations of this report reflect those of the national experts involved in the meetings on aspartame and do not necessarily represent the views of EFSA.

Conclusion

The SCF concluded in 2002 that high-level consumers, both adults and children, were unlikely to exceed the ADI of 40 mg/kg bw per day for aspartame. Consumption by subgroups such as diabetics who are likely to be high consumers of foods containing aspartame were also well below the ADI. The data on aspartame exposure since 2001 confirm the SCF conclusions of 2002 and the National Experts conclude that there are no indications that a population group could exceed the ADI for aspartame.

2. Brain Function

The review relating to Brain Function includes reports on the direct and indirect cellular effects of aspartame or its metabolites on the nervous system including neurotoxicity and functional aspects published or accessible after 2002. The SCF Opinion of 2002 had in particular considered possible neurological effects of aspartame, in the light of new reports (up to 2002) on the consumption of aspartame in relation to the onset of brain tumours and seizures, headaches, allergies, and changes in behaviour, mood and cognitive function. This review therefore considered any new information available in these areas. No reliable new publications were identified supporting enhanced susceptibility to seizures or induced pathological lesions related to aspartame consumption. The issue of brain tumours is considered in the section on Carcinogenicity (see page 41). Table 2.1 include summaries of new *in vitro*, animal studies and human studies relevant to brain function.

2.1 Summary Table: Brain function

Study Title	Summary of details / specifics of study	Comments on significance
Synergistic interactions between commonly used food additives in a developmental neurotoxicity test. Lau et al. Toxicol. Sci (2006)	Inhibition of differentiation of a mouse neuroblastoma cell line (measured as neurite outgrowth) *in vitro* by direct exposure to four food additives including aspartame.	Direct *in vitro* exposure of neural cells does not take proper account of *in vivo* metabolism both due to extensive and rapid hydrolysis of aspartame in the gut and cellular metabolism (see Discussion).
The effect of aspartame metabolites on human erythrocyte membrane acetylcholinesterase activity Tsakiris et al. Pharmacol. Res. (2006)	After incubation of erythrocyte membranes with aspartame metabolites at concentrations corresponding to aspartame intakes of 10, 18, 34, 150 and 200 mg/kg bw a 57% inhibition of enzyme (AchE) activity was seen with the highest dose.	The dose of 34 mg/kg bw reflects the metabolites production corresponding to the 99%-ile of the daily intake. Consequently, the 57% inhibition of the acetylcholinesterase activity observed at the highest dose level (200 mg/kg bw) is considered to be irrelevant.
The effect of aspartame metabolites on the suckling rat frontal cortex acetylcholinesterase. An *in vitro* study. Simintzi et al Food and Chemical Toxicology (2007)	The effects of the sum of the three aspartame metabolites on acetycholinesterase activity was determined both in the suckling rat frontal cortex and in the pure enzyme at concentrations suggested to be present in the human cerebrospinal fluid after ingestion of 10, 34, 150, 200mg/kg bw aspartame. A significant and concentration dependent decrease of the	The incubation of the sum of metabolites at concentrations suggested to be present in human cerebrospinal fluid after the possibly relevant dose (34 mg/kg bw) resulted in significant decrease of the enzyme activity in rat frontal cortex. When metabolites were assayed separately all elicited significant decrease of the enzyme activity, with methanol and aspartate more potent than phenylalanine.

	enzyme activity of the rat frontal cortex was observed when the three metabolites were simultaneously incubated at the concentrations corresponding to 34 mg/kg bw or higher. When each of the aspartame metabolites were separately assayed, a significant and concentration dependent decrease of the enzyme activity was also observed, for the three concentrations of methanol and aspartate, and only for the two higher concentrations for phenylalanine.	
L-cys and GSH restore the modulation of rat frontal cortex Na^+ K^+ -ATPase activity induced by aspartame metabolites Simintzi et al. Food Chem. Toxic (2008)	Incubation of rat frontal cortex homogenate or pure Na^+ K^+ -ATPase with aspartame metabolites at concentrations expected in the cerebrospinal fluid (CSF) after aspartame consumption of 34, 150 or 200 mg/kg bw, decreased the frontal cortex enzyme activity by 33%, 53% or 57%, respectively, whereas the activity of the pure enzyme was markedly stimulated. When the metabolites were separately incubated with the cortex homogenate, only methanol inhibited Na^+ K^+ -ATPase activity, while aspartate and phenylalanine elicited a significant increase.	The authors concluded that, at least *in vitro*, common detoxification through L-cys and GSH effectively restored normal Na^+ K^+ -ATPase enzymatic activities. From the results it can be inferred that the putative reactive compounds responsible for the inactivation of the enzyme can be scavenged by the thiol groups of glutathione and cystein.
Chronic aspartame affects T-maze performance, brain cholinergic receptors and Na^+ K^+ -ATPase in rats Christian et al. Pharmacol, Biochem. Behav. (2004)	250 mg/kg bw/day aspartame in drinking water of male rats. Rats tested every 2 weeks in a T-maze test with a chocolate as a reward. Muscarinic cholinergic receptor density significantly higher in several areas of brain. Na^+ K^+ -ATPase activity increased in mid brain	The delay in the rats finding the chocolate reward may be biased by organoleptic changes induced by aspartame. This study was not performed according to OECD guidelines and the conclusions, based on one single test and dose are not considered significant.
Effect of chronic methanol administration on amino acids and monoamines in retina, optic nerve, and brain of rat. González-Quevedo et al. Toxicology and Applied Pharmacology (2002)	An experimental study in Sprague-Dawley rats previously depleted of folates with methotrexate and subjected to repeated methanol administration at levels of 2g/kg bw/day. Methanol was given intraperitoneally daily for 2 weeks. The rats showed	Dosing by the intraperitoneal route bypasses liver metabolism and the dose administered in this study (50 times the ADI for aspartame) makes the study outcomes not relevant for human dietary exposure.

	significant blood increase of formate after methanol administration. Methotrexate administration produced a three fold rise of formate. Aspartate was significantly increased in the optic nerve of those animals treated with methotrexate and methanol.	
Aspartame: a safety evaluation based on current use levels, regulations, and toxicological and epidemiological studies Magnuson et al. Crit Rev Toxic (2007)	Review of all literature until the beginning of 2007 No evidence of deleterious effects of aspartame consumption with respect to brain function.	Estimated daily intakes, metabolism, biochemical effects and safety evaluation are critically reviewed.
Direct and indirect cellular effects of aspartame on the brain Humphries et al. Eur. J. Clin Nut (2008)	Review on possible damages and effects (mental disorders, compromised learning and emotional functioning) caused by metabolites of aspartame and a discussion about possible affected metabolic/physiological pathways	Roles of aspartame metabolites are discussed in general terms. Association with neurological and behavioural disturbances in sensitive individuals are based upon two non-peer reviewed internet sources which is considered as not scientifically robust.
Aspartame effects on the brain. (letter relating to Humphries review) Fernstrom Eur. J. Clin Nut (2008)	This published letter provides a critical opinion on the 2008 review by Humphries et al. (2008) Fernstrom concluded that the intraperitoneal injections of aspartame of about 2 g/kg bw are unrealistic for humans and that relevant scientific findings (e.g. the authors are incorrect in stating that tyrosine can not be synthesized in the brain from phenylalanine) were omitted. (see also comments on González-Quevedo et al. 2002)	Fernstrom notes that nine scientific errors are erroneously repeated, are given undue weight, and subsequently incorrectly enhance the data leading to unsubstantiated as well as misleading interpretations.
Seizures and Hyponatremia after excessive intake of diet coke Mortelmans et al. Eur. J. Emerg. Med. (2008)	A 54-year-old female suffering a status epileticus after ingestion of 9L diet coke. She recovered after intravenous correction for hyponatremia, forced diuresis and delirium prevention. Postconvulsive rhabdomyolosis was diagnosed	Seizures were clearly the result of a hyponatremic condition, according to the authors, possibly potentiated by caffeine and/or aspartame. (Rhabdomyolosis can be induced by hyponatremic seizures and caffeine overdose).

Discussion

The objective of the Lau et al. (2006) study was to test neurotoxicity *in vitro* using the endpoint of differentiation of a mouse neuroblastoma cell line, measured as neurite outgrowth. The study was performed based on the assumption of synergistic inhibition effects of additives, including aspartame.

The results indicated that aspartame inhibits neurite outgrowth in neuroblastoma cells *in vitro*. The authors concluded that the NMDA receptor plays a role in the effect and that the effect is independent of cytotoxicity. Aspartame has an IC50 of 153 μM in the system studied, in which neurite outgrowth is monitored 24 hours after changing cells to serum free conditions plus test compound(s). Synergism of aspartame with the food additive Quinoline Yellow is demonstrated in the study but remains unexplained at the mechanistic level. The authors compare the IC50 of aspartame with calculated potential human serum concentrations after beverage consumption (58 μM) and concluded that they are in the same order of magnitude.

Lau et al. (2006) remark that the *in vitro* system may be more susceptible than an *in vivo* model. The Organising Team considers that *in vitro* tests can be useful for obtaining mechanistic information as well as for regulatory screening purposes for prioritization. However, direct extrapolation from *in vitro* to *in vivo* situations should also in the case of aspartame include the following considerations:

- aspartame undergoes extensive and fast hydrolysis in the gut; therefore, an *in vitro* test would need to take into account the metabolic products from this process and not aspartame itself;

- Phase I and Phase II metabolism by the liver;

- toxicokinetics and pharmacodynamic characteristics need to be considered;

- blood: brain barrier considerations;

- cellular and species specificity in metabolism;

The Organising Team additionally noted that the basis of the culture system was serum-free medium, and that serum-free cultures can be more susceptible to compounds, due in particular to limited protein binding. The IC50 in this assay cannot be extrapolated to the *in vivo* situation and needs to be considered alongside the extensive information available on *in vivo* kinetics of aspartame at doses close to the ADI. The principal drawback of this study is the direct incubation of aspartame with the neuroblastoma cell line, because it is already very well proved that aspartame is extensively metabolized in the gut and, consequently, it is expected that only metabolites can reach the brain. Therefore this study is considered irrelevant.

Tsakiris et al. (2006) reported on a dose-dependent reduction of acetylcholinesterase activity caused by aspartame metabolites using erythrocyte membranes as a model. Metabolite concentrations used were much higher than those documented for plasma samples after an estimated aspartame consumption of 34 mg/kg bw per day (suggested by the authors to correspond to the 99%-ile daily intake). Consequently, the 57% inhibition of the acetylcholinesterase activity observed at the highest dose level (200 mg/kg bw) is likely to be irrelevant.

Simintzi et al, (2007), showed a significant and concentration dependent decrease of the acetylcholinesterase activity in the suckling rat frontal cortex after incubation with concentrations of the three metabolites corresponding to those expected after intake of aspartame at the doses of 34, 150, or 200 mg/kg. When the metabolites were incubated separately with rat frontal cortex, it was observed that methanol and aspartate were more potent than phenylalanine in eliciting the inhibition of the enzyme activity. Although in vitro, these results show that the metabolites originated at the relevant dose of 34 mg/kg/day (stated by Tsakiris et al. (2006) to correspond to the 99%-ile daily intake) are inhibitors of acethylcholinesterase to a significant extent.

In 2008, Simintzi et al. (2008) observed a significant and concentration dependent decrease of Na^+/K^+-ATPase after incubation of frontal cortex homogenate with aspartame metabolites at concentrations

expected after ingestion of aspartame at doses of 34 mg/kg bw or higher. When the metabolites were incubated separately, only methanol elicited significant inhibition while aspartate and phenylalanine significantly increased the enzyme activity.

Interestingly, in this *in vitro* model, L-cysteine and glutathione could restore the Na^+ /K^+ -ATPase enzymatic activities to normal levels, indicating that reactive species could be responsible for the enzyme inhibition (for example formaldehyde, metabolite of methanol, an electrophilic species).

The assessment of aspartame effects on behaviour and learning abilities of Sprague-Dawley rats was performed by Christian et al. (2004). Aspartame was administered in water at a concentration of 250 mg/kg bw/day for 120 days and the time taken to find a reward (chocolate) in a T-maze test was measured. Memory loss was associated with change in density of muscarinic cholinergic receptors in several brain areas. In contrast with some data in the *in vitro* studies referred to above, additionally significantly higher levels of Na^+ /K^+ -ATPase activity were found in the midbrain. Administration of only one dose and the use of just one T-maze test were considered by the Organising Team to be limitations of this study. Furthermore, it was not possible to conclude if delayed times taken to find the reward were due to satiation of desire for sweetness as aspartame was administered in drinking water: thus chocolate may not have been an appropriate reward. In a recent review by Magnuson et al. (2007) it is stated that "well designed studies using a range of approaches to evaluate learning and memory consistently demonstrate no effect of aspartame consumption at levels up to 4000 mg/kg/day".

Repeated i.p. administration of methanol or methanol plus methotrexate (to decrease the hepatic tetrahydrofolate concentration) at 2g/kg/day methanol for two weeks, significantly increased the hippocampus levels of aspartate, glutamate, glutamine, and taurine, as well as an increase of aspartic acid levels in the optic nerve (González-Quevedo et al. 2002). Some of these amino acids are recognized excitotoxic compounds, like aspartic acid and glutamic acid. It is important to highlight that a consistent increase was observed in the hippocampus, a determinant brain area for memory. Methanol administration also altered the retinal and hippocampal serotonergic system, as shown by the significant increase of 5-hydroxytryptamine content in the retina and hippocampus. The dose of methanol administered to the rats is unrealistic when compared with the ADI of 40 mg/kg bw per day for aspartame, making the significance of the obtained results very questionable.

Taken together, these experiments performed both *in vitro* and *in vivo* suggest an interference by the aspartame metabolites in some important biochemical pathways in the brain. Due to the highly complex nature of the biochemical and molecular pathways in the brain, the Organising Team considers that animal studies are more informative than *in vitro* studies in shedding light on the effects of aspartame metabolites in an integrative manner, including the influence in the protein expression, ionic gradients, and neurotransmitters level. Additionally, defining putative toxicity mechanisms such as oxidative stress, loss of calcium homeostasis, and type of cell death is of importance.

The Institute of Medicine (2005) questioned the neuronal necrosis effects observed in newborn rodent models as indicative of possible similar effects in humans in utero due to high levels of aspartic acid. The effects were observed on newborn rodents with high plasma levels of phenylalanine and aspartic acid, but not in human newborns or in non-human primate newborns under similar exposure conditions.

The only reference to seizures and hyponatremia after excessive intake of diet coke by a 54-year-old female is not of relevance, being a case report only one individual with a large aspartame intake (9 l of diet coke) (Mortelmans et al. 2008).

Conclusion

Several studies, *in vitro* or *in vivo*, indicate that aspartame or its metabolites may affect certain enzyme activities in the brain, for example acetylcholinesterase (such as Simintzi et al. 2007), Na^+/K^+-ATPase (Christian et al, 2004, Simintzi et al, 2008) or cytochrome P450 (CYP) enzymes (e.g. Tutelyan et al, 1990, discussed in the section on metabolism). The National Experts consider that the biological relevance of such findings is not clear, particularly the relevance of findings in *in vitro* studies in which the toxicokinetic and toxicodynamic behaviour of aspartame *in vivo* is not fully reflected. The National Experts consider however that the scientific literature needs to be monitored for further research and mechanistic explanations related to this area.

The National Experts note that no new publications were identified reporting a link between aspartame intake and enhanced susceptibility to seizures, behaviour, mood and cognitive function, and concludes that there is still no substantive evidence that aspartame can induce such effects, as earlier concluded by the SCF.

3. Effects on Satiation and Appetite

All papers since 2002 describing effects of aspartame on enhancement or suppression of appetite and the state of satiation have been considered in this section. In some reports the effects of aspartame were not being studied as aspartame was being used as a control. Some relevant findings from studies on 'sweeteners' are presented below. However in these studies aspartame was given as part of a mixture and it is acknowledged that the evidence from these studies cannot be considered to be specifically relevant to aspartame.

A total of 49 post-2002 publications were identified for consideration. Several papers identified were surveys reporting intake of aspartame, and some were reviews so did not report novel findings. Some papers did not report findings of studies on aspartame, but referred to it in discussion. These were not considered further.

3.1 Summary Table: Satiation and Appetite

Study Title	Summary of details / specifics of study	Comments on significance
Effects of a sweet and a nonsweet lunch on short-term appetite: differences in female high and low consumers of sweet/low-energy beverages Appleton et al. J Hum Nut Diet. (2004)	Study looking at appetite in 3 groups of women, those who habitually consume low energy drinks, sweetened low energy drinks and sweet sugar drinks.	Small study, 8 in each group. Suggests "short-term control of appetite varies according to habitual pattern of dietary intake. Long-term experience of sweetness without energy influences appetite for sweet and savoury tastes." Sweeteners in general not specifically aspartame studied
Habitual high and low consumers of artificially-sweetened beverages:	Suggested that low level consumers of sweeteners tended to have their appetite affected more by sweeteners than habitual consumers.	Suggests variation according to habitual intake.

Effects of sweet taste and energy on short-term appetite Appleton and Blundell Physiol. & Behav.(2007)		Sweeteners in general, not specifically aspartame studied
Effects of long-term ingestion of aspartame on hypothalamic neuropeptide Y, plasma leptin and body weight gain and consumption Bech et al. (2002)	Rats administered aspartame in drinking water (1%) for 14 weeks. Observed lower body weight and fat depot weight in treated animals, with no differences in energy intake or plasma insulin levels. Plasma leptin significantly reduced.	Well conducted rat study (n=24). Authors suggest beneficial effects on body composition could be due to decreased effects of neuropeptide Y on lipid and energy metabolism, independent of insulin.
Effects of Energy-Content Labels and Motivational Posters on Sales of Sugar- Sweetened Beverages: Stimulating Sales of Diet Drinks among Adults Study Bergen and Yeh. J Am Dietetic Assoc. (2006)	Study looking at a public health approach to reduce energy intake.	Authors suggest a message will encourage a change in behaviour. No link to effect on appetite.
Sugar-sweetened and artificially sweetened soft drinks in association to restrained, external and emotional eating Elfhag et al. Physiol. & Behav. (2007)	Survey data of use of soft drinks	Suggests that sweet drinks might be used as a comfort, no link to appetite or physiology Sweeteners in general not specifically aspartame studied
The Effect of Increased Beverage Portion Size on Energy Intake at a Meal Flood. J Am Dietetic Assoc. (2006)	Looking at the effect of giving a larger drink on actual intake. If you offer a bigger drink, subjects drank more. If drink contained energy then energy intake increased.	No difference in food intake between water and diet drink. Study not designed to look at effect of aspartame on appetite, satiety or weight gain. Sweeteners in general not specifically aspartame studied
Short-term effects of chewing gum on snack intake and appetite Hetherington and Boyland. (2007)	Looked at the effect on appetite and desire for sweet foods, eating or not eating gum	Subjects choose favourite gum, not comparison of regular and sugar free gums, no clarity of sweeteners used or consumed.
Influence of sweetened chewing	Well designed study, acknowledges	A no effect study. No control gum,

gum on appetite, meal patterning and energy intake Julis. Appetite (2007)	weaknesses of a lack of control sweetened gum, (assumed to contain sugar, corn syrup, sucralose and aspartame).	not specifically looking at aspartame. A previous study cited suggested an effect, including a biphasic effect on appetite of high dose aspartame. Initially decreasing and then increasing appetite (Tordoff and Alleva 1990 Physiological Behaviour 47 – 555-9) Sweeteners in general not specifically aspartame studied
Artificial Sweetener Use among Individuals with Eating Disorders Klein et al. Int J Eating Disorders (2006)	Survey of consumption of artificially sweetened foods. Reports a greater use of sweeteners amongst those with eating disorders (particularly binge eating/ purging) than controls.	No causal association that binge eating is linked to sweetener use, purely an observation. Sweeteners in general not specifically aspartame studied
Effects of hunger on flavour pleasantness conditioning at home: Flavour-nutrient learning vs flavour-flavour learning Mobini et al. Appetite (2007)	A study of perception of "sweetness" and "pleasantness" of drinks (artificially sweetened, sucrose sweetened or minimally sweetened) depending on state of hunger.	Does not look at effects of aspartame on appetite or satiation but at how 'pleasant' sweet drinks are perceived to be when hungry/sated.
Long-term dietary compensation for added sugar: effects of supplementary sucrose drinks over a 4-week period Reid et al. Br J Nut. (2007)	A study looking at effect of sucrose supplementation on appetite. Concludes that sucrose supplementation moderates the diet.	Aspartame is not actually being studied but is given as placebo as such. The paper does not assess change in their behaviour when consuming aspartame.
First European conference on aspartame: Putting safety and benefits into perspective. Synopsis of presentations and conclusions Renwick and Nordmann. Food and Chem Tox. (2007)	Review of evidence of risk and benefit. States replacement of sucrose with aspartame could result in decrease calorie intake and from a meta-analysis adequate prolonged weight reduction could be achieved with aspartame.	
Influence of repeated consumption of beverages containing sucrose or intense sweeteners on food intake van Wymelbeke et al. Eu J Clin	Influence of the ingestion of beverages sweetened with sucrose or artificial sweeteners on food intake on hunger ratings in males and females.	Small study (n=24). Observed no adjustment of caloric intake when consuming high energy drinks, no change in hunger ratings with either drink.

Nut. (2004)		
		Aspartame not studied alone but as a mixture with Acesulfame K and Saccharin.

Discussion

A number of studies focused on the effects of aspartame on appetite/hunger and food intake (Appleton and Blundell (2007), Reid et al. (2007), Appleton et al. (2004), Van Wymelbeke et al. 2004) as it has been suggested that aspartame may have modulating effects on these body responses, even resulting in the converse effect than that intended, namely obesity rather than body weight maintenance or loss. It is suggested that the experience of sweet taste without a calorific intake has an influence on appetite for both sweet and savoury foods. It should be noted, that in the majority of these studies aspartame was included as part of a mixture of sweeteners. No studies focused on the effects of aspartame on body weight.

There is little or no substantive data suggesting that aspartame may adversely rather than positively affect obesity in consumers. In considering the continued increase in the obesity of the population and that more consumers may potentially be turning to calorie free sweeteners and use of calorie free sweeteners, Just et al. (2008) looked at cephalic insulin response in healthy fasting volunteers after taste stimulation, comparing sucrose, starch and saccharin and reported that a significant increase of plasma insulin concentration was apparent after stimulation with both sucrose and saccharin. This result suggests that sucrose and saccharin activate a cephalic phase insulin response when applied to the oral cavity only. A similar study focusing on aspartame warrants further consideration.

Interpreting the data to answer the specific question regarding whether aspartame has a direct effect on appetite is difficult, as this was not the hypothesis of the identified studies. Whilst it is encouraging that no trend of increasing appetite has been observed, the question is still largely unanswered, but remains an important one. This may be of specific relevance to particular subject groups. For example, whether aspartame may have an effect depending on Body Mass Index (BMI) or co-morbidities such as diabetes has not been investigated.

Conclusion

The National Experts note that there is little or no substantive data suggesting that aspartame affects appetite/hunger, food intake. A study focusing on aspartame, such as that performed by Just et al. (2008) which looked at cephalic insulin response in healthy fasting volunteers after taste stimulation, comparing sucrose, starch and saccharin, warrants further consideration. Studies to answer this question could address appetite in groups with stratified BMIs, those in whom obesity and insulin resistance are co-morbidities, such as impaired glucose tolerance and type 2 diabetes. It should also be determined if aspartame alters exercise modified metabolic responses. Such studies should be performed using food or drink products which would have to be designed with appropriate consideration to blinding.

4. Allergenicity and Immunotoxicity

In this section publications from 2002 dealing with possible immunotoxicity and allergenicity of aspartame have been evaluated.

4.1 Allergenicity

Adverse reactions to foods that have an immunological basis are termed 'food allergies'. However, adverse reactions to food not only include food allergies, but may also result from reactions that do not involve the immune system. The different causes of adverse reactions to food can be summarized as follows:

- toxic reactions: non immune system-mediated reactions, but food poisoning by toxins or by bacterial, viral or parasitic contamination
- food aversion: non immune system-mediated reactions, but psycho-somatic effects such as panic disorder)
- hypersensitivity reactions divided into:
  - food intolerance: non immune system-mediated reactions, but due to e.g. enzymatic deficiencies (e.g. lactase intolerance), pharmacological effects (e.g. to caffeine, histamine, tyramine), and still undefined mechanisms, and
  - food allergy: immune system-mediated reactions such as Immediate-Type Hypersensitivity (ITH), which is a Type I, IgE-mediated hypersensitivity, like in the majority of food allergies (e.g. to cow's milk, hen's egg, peanut, tree nuts, soy, fish, shell-fish and seafood etc.) or a Delayed-Type Hypersensitivity (DTH), which is a Type IV, effector T cell-mediated reaction, like in the gluten intolerance syndrome (Coeliac disease).

Although it is clear that the cause of the adverse reactions to food can be different, clinical features can in part be similar such as various gastrointestinal complaints.

Food allergy is therefore defined as an immunologicallly-mediated adverse reaction to food (mainly to food proteins) that results in allergic sensitization, and upon subsequent exposure to the offending food in local or systemic allergic hypersensitivity reactions. The clinical symptoms of allergic hypersensitivity reactions may include skin (urticaria), oral (itching and swelling of the mouth), gastro-intestinal (vomiting, diarrhoea), respiratory (wheezing, rhinitis, asthma), and cardiovascular (anaphylaxis) complaints.

Concerning the potential allergic-type reactions resulting from aspartame ingestion no publications were found after 2002 dealing with novel findings. Only two review papers have been published (Butchko et al. 2002, Magnuson et al. 2007), in which the subject of allergy-type reactions to aspartame were discussed and summarised. Moreover, no reports have been published referring to food intolerance reactions attributed to aspartame intake.

4.1 Summary Table: Allergenicity

Study Title	Summary of details / specifics of study	Comments on significance
Aspartame: review of safety Butchko et al. Reg Toxicol	The paper reviews the safety of aspartame including the allergic-type reactions attributed to	The authors concluded that the weight of evidence demonstrates that aspartame is not associated

Pharmacol. (2002)	aspartame.	with allergic-type reactions in experimental models or humans
Aspartame: A safety evaluation based on current use levels, regulations and toxicological and epidemiological studies. Magnuson et al. Crit Rev Toxicol. (2007)	This paper reviews the safety of aspartame but no new points were raised concerning allergic-type reactions other than those already discussed in the review of Butchko et al.	This paper also refers to the same published papers as discussed by Butchko et al (2002) and in the SCF (2002) opinion. They conclude that the allergic-type reactions belong to the category of potential effects of which the available evidence is not adequate to conclude that the reported effect actually exists.
Case report: Formaldehyde, Aspartame, and migraines: A possible Connection. S.E. Jacob and S.Stechschulte. Dermatitis (2008)	This paper discusses the possible connection between aspartame-associated migraines and dermatitis due to the aspartame metabolite formaldehyde in patients suffering already from delayed type hypersensitivity reactions to formaldehyde.	This case report refers to six patients. The authors conclude that a larger case study is needed to firmly establish the association between aspartame breakdown products, migraine, systemic contact dermatitis, and positive patch test reactions to formaldehyde.
Case report: Systemic contact dermatitis of the eyelids caused by formaldehyde derived from aspartame. A.M. Hill and D.V. Belsito Contact Dermatitis (2003)	This paper discusses the possible connection between aspartame intake, especially the aspartame metabolite formaldehyde, and eyelid dermatitis in a patient with proven contact sensitization to formaldehyde.	The authors conclude that because the patient refused to undergo a re-challenge with aspartame, which could have proven that her hypersensitivity was due to aspartame intake and subsequent metabolism resulting in e.g. formaldehyde and formaldehyde adducts, the relationship remains speculative.

Discussion:

Butchko et al. (2002) reviewed all published papers from 1980 onwards reporting allergic-type reactions attributed to aspartame exposure. In an evaluation of consumer complaints related to aspartame by Center for Disease Control and Prevention (CDC) (Food and Drug Administration, 2010) approximately 15% of the anecdotal complaints were assigned to allergic-dermatologic reactions attributed to aspartame ingestion, such as rashes, sore throat/mouth, swelling and itching. Furthermore case reports exist of urticaria (Kulczycki, 1986) and granulomatous panniculitis (Novick, 1985, McCauliffe and Poitras, 1991) which were all thought to be related to aspartame and were previously considered by the SCF (2002).

Limitations of these single case reports were highlighted by Geha et al. (1993, 1995). They conducted a multi-center placebo-controlled clinical study to evaluate individuals who had experienced clinical signs associated with allergy. In a double-blind crossover study recruited subjects with a likely history of hypersensitivity to aspartame were exposed to aspartame and placebo. The authors concluded that aspartame and its conversion products were no more likely than placebo to cause allergic symptoms in the subjects. These conclusions were further confirmed in a study by Garriga et al. (1991). They also concluded that after a single blind and double blind placebo controlled food challenge (DBPCFC) with

aspartame exposures up to 2000 mg none of the clinically evaluated and subsequently challenged subjects exhibited clear reproducible adverse reactions to aspartame.

Studies of Szucs et al. (1986) concluded that neither by *in vitro* methods nor by skin prick testing (SPT) *in vivo* aspartame was found to induce a direct degranulation of mediators from mast cells or basophils which will result in allergy-type reactions. No additional data were available for consideration by the SCF in 2002 or more recently by Magnuson et al. (2007).

More recently some case reports have been published (Jacob and Stechschulte, 2008, Hill and Belsito, 2003) in which associations are made between aspartame intake, in particular the subsequent exposure to the aspartame metabolite formaldehyde, and Type IV Delayed Type Hypersensitivity (DTH) reactions in patients with proven contact sensitization to formaldehyde. However, to determine whether the associations suggested by these two case studies with only a limited number of patients are established, larger case studies are needed with double-blind placebo-controlled challenges using aspartame and placebo exposures, which should also include well defined control patient groups.

4.2 Immunotoxicity

Immunotoxicology research is focused on studying the unwanted and specific effects of industrial chemicals, food ingredients/supplements and drugs on the immune system. Assessment of immunotoxicity does not differ from the assessment of toxicities in other organ systems. It studies the effects of compounds on organs, tissues and cells of the immune system and its consequence for the function of the immune system. If repeat-dose toxicity studies demonstrate that a test compound induces effects on organs, tissues or cells of the immune system additional immune function studies may be requested. The type of immune function study to be conducted will largely depend on the nature of the effects and may comprise studies focused on specific immunity (e.g. cell-mediated immunity (DTH) or humoral immunity), non-specific immunity (e.g. Natural Killer (NK) cell activity, macrophage or neutrophil activity) or host resistance models (e.g. resistance to a bacterial/viral infection).

In respect to the potential immunotoxicology of aspartame five papers were evaluated. These papers mainly focused on the effects of metabolites of aspartame particularly in *in vitro* studies using immune system-related thymocytes and *in vivo* tests using various immune function endpoints.

4.2 Summary Table: Immunotoxicity

Study Title	Summary of details / specifics of study	Comments on significance
Formaldehyde-induced shrinkage of rat thymocytes. Nakao, et al. J Pharmacol Sci (2003)	The effects of the aspartame metabolite formaldehyde was studied *in vitro* on shrinkage of thymocytes	The *in vitro* study used concentrations of aspartame metabolites, in particular formaldehyde, not likely to occur *in vivo*. Therefore the *in vitro* observed effects on thymocytes are not considered to be relevant for the *in vivo* situation.
Cytotoxic effects of methanol, formaldehyde, and formate on dissociated rat thymocytes: a possibility of aspartame toxicity. Oyama, et al. Cell Biol Toxicol (2002)	The effects of aspartame metabolites (methanol, formaldehyde and formate) were studied *in vitro* on various endpoints in thymocytes, viz. viability, apoptosis and Ca^{2+}-influx.	As the *in vitro* used concentrations of aspartame metabolites, in particular formaldehyde, are not likely to occur *in vivo*, the *in vitro* observed effects on thymocytes are not considered to be relevant

		for the *in vivo* situation.
Effect of methanol-induced oxidative stress on the neuron-immune system of experimental rats. Parthasarathy et al. Chem Biol Interactions (2006)	*In vivo* effects of methanol were studied in rats at ¼ of the minimal lethal dose resulting in impairment of the hypothalamus-pituitary-adrenal axis. Next to changes in corticosteroid levels effects on immune functions were measured (CMI decreased, HI increased)	The methodologies used to assess most immune system-related endpoints were not based on the current status of immune-toxicity testing. Moreover, due to the high dose level of methanol used the observed effects on lymphoid organs, cells and function may rather be due to stress than to a direct effect of methanol. Furthermore, the methanol dose used is also considered not to be of relevance in respect to aspartame consumption
Effects of methanol intoxication on specific immune functions of albino rats. Parthasarathy et al. Cell Biol Toxicol (2007)	*In vivo* effects of methanol were studied in rats at ¼ of the minimal lethal dose. Various immune system-related endpoints were found to be affected and both humoral and cell-mediated responses were decreased.	The methodologies used to assess most immune system-related endpoints were not based on the current status of immune-toxicity testing. Moreover, due to the high dose level of methanol used the observed effects on lymphoid organs, cells and function may rather be due to stress than to a direct effect of methanol. Furthermore, the methanol dose used is also considered not to be of relevance in respect to aspartame
Possible analgesic and anti-inflammatory interactions of aspartame with opioids and NSAIDs. Sharma et al. Indian J Exp Biol (2005)	The study indicated that aspartame exerted analgesic and anti-inflammatory effects on its own and has a synergistic analgesic response with conventional analgesics of opioid and non-opioid type.	The study is not really relevant for the evaluation of the potential immunotoxicity of aspartame and its quality is considered poor.

Discussion:

In a study by Oyama, et al. (2002) the *in vitro* effects of the aspartame metabolites methanol, formaldehyde and formate on rat thymocytes were reported. Based on the results obtained it was shown that the viability of thymocytes was not affected by methanol up to 100 mmol/L, or by formate up to 30 mmol/L , but a significant effect was seen with formaldehyde at concentrations of 3-30 mmol/l. Additional studies with formaldehyde showed that apoptosis (programmed cell death) of thymocytes occurred at significantly higher concentrations of 10 mmol/L and above; the Ca 2+ influx increased significantly from 3 mmol/L and the glutathione content decreased from 1 mmol/L.

The authors concluded that formaldehyde from concentrations of 1 mmol/L was toxic for thymocytes *in vitro*.

It is unlikely that the formaldehyde concentration will reach 1 mmol/L *in vivo* (Stegink et al. 1981) as it is rapidly metabolised or will be bound to tissue proteins (Matsumoto et al. 1990). The authors concluded that because the blood concentration of formaldehyde will probably be lower than that of methanol after aspartame ingestion and the blood methanol concentrations in subjects administered

150-200 mg/kg body weight of aspartame was less than 1 mmol/L, even at high doses aspartame appears harmless to humans in respect to these observed effects on thymocytes *in vitro*.

Nakao, et al. (2003), reported the *in vitro* effects of the aspartame metabolite formaldehyde on shrinkage (an early marker for cytotoxicity) of rat thymocytes. It was shown that formaldehyde induced a dose-dependent decrease (100-300 μM) in the number of normal sized cells and an increase the number of shrunken cells. The authors concluded that at a blood formaldehyde concentration of 100 μM or greater there would be a decrease in the population of intact thymocytes, This is unlikely for several reasons: it is not likely that blood formaldehyde concentration of 100 μM or more will be attained by aspartame ingestion and the weight and histopathology of the thymus has not been shown to be affected by aspartame in repeat-dose studies in rodents.

Sharma et al. (2005) investigated the analgesic and anti-inflammatory properties of aspartame alone and in combination with various opioids and non-steroidal anti-inflammatory drugs (NSAIDs), with respect to a possible synergistic effect. The study not considered relevant for evaluation of possible immunotoxicity of aspartame and its quality is poor.

Parthasarathy et al. (2006, 2007) studied the *in vivo* effects of methanol, a known metabolite of aspartame in male rats at levels of 2.37 g/kg bw/day administered intraperitoneally for 1, 15 and 30 days (minimal lethal dose of methanol is 9.5 g/kg bw/day in rats). In their first study (2006) they reported that methanol intoxication resulted in effects on the antioxidant status, lipid peroxidation and DNA integrity in the hypothalamic-pituitary-adrenal axis. In addition effects on the non-specific and specific immune functions were analysed resulting in decreased cell-mediated immune responses as measured by Delayed Type Hypersensitivity (DTH) and increased leukocyte migration inhibition. The humoral immunity (antibody titers) was reported to be increased. Plasma corticosterone levels were also found to be increased in day 1 and day 15 animals but decreased in day 30 animals

The conclusion of the authors was that methanol toxicity accelerates free radical activities resulting in an unbalanced antioxidant status in the hypothalamus and adrenal glands. The altered hypothalamic-pituitary-adrenal axis impairs the corticosterone levels in the blood stream, which affected neutrophil function, cell-mediated and humoral immune response.

In the second study of Parthasarathy et al. (2007) the *in vivo* effects of methanol intoxication were studied using the same exposure protocol as used in the first study (2006). On day 15 and day 30 significant decreases in lymphoid organ weight ratio's, lymphoid cell counts, footpath thickness (DTH), antibody titers (in their first study of 2006 the antibody titers were increased), several cytokines and several lymphocyte subsets (T and B cells) were observed. Plasma corticosterone levels were increased in day 15 animals but decreased in day 30 animals.

The conclusion of the authors was that the results of the present study also showed that methanol toxicity produced a reduction in antibody titers, footpath thickness, cytokine production and lymphocyte subsets, so methanol markedly suppressed both humoral and cell-mediated immune responses.

It is questionable whether the observed effects in both Parthasarathy studies (2006, 2007) on organs/tissues/cells and on function of the immune system were due to direct effects of the aspartame metabolite, methanol. The effects seen would appear to result indirectly, thus being secondary to the methanol-induced stress. This was also suggested by the authors in their first study which summarised ''the disturbed hypothalamic-pituitary-adrenal axis alters the level of corticosterone, which leads to varied non-specific and specific immune responses in experimental rats''.

Furthermore, the methanol levels used are not considered relevant for aspartame exposure, the quality

of the study (methodologies used) was poor for some measured endpoints, and the result questionable (humoral immunity was increased in first study and decreased in second study using the same experimental design). In summary, it is possible to conclude that the observed immunotoxic effects of the aspartame metabolite methanol do not appear to be due to a direct immunotoxic effect of methanol, but are more likely to be due to an indirect effect resulting from methanol-induced stress.

These studies on the potential immunotoxicity of aspartame metabolites, provide new information which was not previously considered in the JECFA/SCF opinions. However, the National experts note the limitations in the data identified and their unknown biological significance.

The *in vitro* observed effects in these studies on rat thymocytes are not considered to be relevant to the *in vivo* situation.

Conclusion

Although anecdotal reports in the early 1980s suggested that aspartame might be associated with allergic-type reactions, several clinical studies have shown that when the allergic-type reactions raised in these case reports were evaluated under controlled conditions, it was clear that aspartame is no more likely to cause reactions than placebo. The weight of evidence collected in the various publications demonstrates that it is not likely therefore that aspartame is associated with allergic-type reactions in experimental models or humans. No reports have been published referring to food intolerance reactions attributed to aspartame intake.

In some recent case reports (Jacob and Stechschulte, 2008, Hill and Belsito, 2003) associations have been made between aspartame intake, in particular the subsequent exposure to the aspartame metabolite formaldehyde, and Type IV Delayed Type Hypersensitivity (DTH) reactions in patients with proven contact sensitization to formaldehyde. However, to confirm the associations observed in these two case studies with only a limited number of patients (7in total), larger studies would be needed involving double-blind placebo-controlled challenges with aspartame and placebo exposures and the inclusion of well defined control patient groups.

Based on the results presented in the publications evaluated it can be concluded that no convincing, scientifically robust evidence has been presented that aspartame or one of its metabolites might be immunotoxic. The prediction of the potential immunotoxic effects of the aspartame metabolite methanol based on *in vitro* studies with thymocytes (Oyama et al. 2002, Nakao et al. 2003) is highly speculative

Although in subsequent *in vivo* studies by Parthasarathy et al. (2006, 2007) effects of the aspartame metabolite methanol on organs/tissues/cells and function of the immune system were described, these observations are considered more likely to result from an indirect stress-effect due to the high methanol levels used, in addition these high dose levels are not considered relevant for aspartame exposure. In addition, the quality of the studies was poor.

The National Experts conclude that there is no new evidence published since 2002 that requires a review of the existing SCF opinion (2002) which stated "Studies on allergic-like reactions in individuals whom themselves reported such reactions to aspartame have not confirmed their occurrence when later studied under controlled conditions'' and there are no reports in the literature reviewed that indicate human aspartame use might be associated with immunotoxic interactions.

5. Effects of aspartame on metabolism of endogenous and exogenous compounds

In the literature reviewed by the Organising Team it has been observed that the metabolites of aspartame (aspartic acid, phenylalanine and methanol) could affect the metabolism of endogenous and exogenous compounds. Amino acids per se have an influence on metabolic pathways and it is known that high doses of aspartame may increase plasma levels of the metabolites of aspartame (Magnuson et al 2007). High levels of specific amino acids can also affect transporters and protein synthesis. The literature search for publications since 2002 relating to this area identified two articles that have been evaluated (Ferland et al. 2007; Fujita et al 2007).

The cytochrome P450 (CYP) system represents an important part of metabolic pathways. An activation of this enzyme system could result in an increased rate or efficiency in the metabolism of xenobiotics (Dutheil et al. 2007). The literature search has identified two articles (Tutelyan et al. 1990; Vences-Meja et al. 2006) that have been evaluated.

5.1 Summary Table: Metabolism

Study Title	Summary of details / specifics of study	Comments on significance
The effect of aspartame on the activity of rat liver xenobiotic-metabolizing enzymes. Tutelyan VA, Kravchenko LV, Kuzmina EE. Drug metabolism and Disposition: The Biological Fate of Chemicals (1990)	In rats, increases in three liver microsomal cytochrome P450 (CYP) activities were measured after 45 days of daily aspartame administration in the diet (40 and 4000mg/kg bw/day), but these effects were normalised after 90 days of treatment.	Consumption of aspartame was shown to cause a transient increase in microsomal liver enzymes of the cytochrome P450 (CYP) family
Is aspartame really safer in reducing the risk of hypoglycaemia during exercise in patients with type 2 diabetes? Ferland A, Brassard P, Poirier P. Diabetes Care (2007)	It was shown that in patients with type 2 diabetes (14 males) reduction of plasma glucose and insulin levels during exercise was similar after a sucrose meal compared to a aspartame sweetened meal	Important to consider since the nutritional requirements during exercise are especially sensitive to imbalances. Small study of 14 individuals
Effects of L-phenylalanine supplementation and a high protein diet on pharmacokinetics of cefdinir in healthy volunteers: an exploratory study. Fujita et al. Diabetes Care (2007)	Phenylalanine supplementation (2.5 g, three times a day for 12 days to 6 healthy male volunteers) did not appear to up-regulate intestinal oligopeptide transport, in terms of the used drug cefdinir.	Phenylalanine at levels above expected human intake does not affect gastrointestinal absorption of oligopeptides
The effect of aspartame on rat brain xenobiotic-metabolizing enzymes. Vences-Meja et al. Hum Exp Toxicol. (2006)	The expression (protein) and activity of several cytochrome P450 (CYP1, 2 and 3) enzymes in brain microsomes was increased in rats administered aspartame by oral gavage (75 and 125 mg/kg bw/day for 30 days), but was not detectable in control rats or in the liver of aspartame treated rats.	Induction of CYP immunoreactive proteins by aspartame paralleled that of increased enzyme activities and these effects were brain specific. A draw-back of the study is that pooled tissue samples were used

Discussion

Metabolism including diabetes:

In the literature prior to 2002 no differences were identified between normal and diabetic individuals in the absorption and metabolism of aspartame, and no subsequent publications suggest such a difference. One new reference showed, in patients with type 2 diabetes, that the reduction of plasma glucose and insulin levels during exercise was similar after a sucrose meal compared to an aspartame sweetened meal (Ferland et al. 2007). These results were obtained even though the aspartame meal contained 22% less calories and 10 % less carbohydrates.

Metabolism – gastrointestinal uptake:

Publications unrelated to aspartame indicate that phenylalanine in the intestine can up-regulate oligopeptide transporters which could result in a changed uptake of nutrients and drugs based on amino acids (peptides). In the paper by Fujita et al. (2007) it is shown that phenylalanine supplementation (2.5 g, three times a day for 12 days in healthy volunteers) did not seem to up-regulate intestinal oligopeptide transport. The substance used was cefdinir, a β-lactam antibiotic with a normally low absorption (33%). Thus, a phenylalanine intake, comparable to the consumer intake of aspartame has not been shown to affect the uptake of peptide-based substances.

Effects on the metabolising system – the cytochrome P450 system:

Two studies on metabolising cytochrome P450 (CYP) enzymes have been identified.

In the study by Tutelyan et al. (1990), not considered in the 2002 evaluation by the SCF, increases in activity of three liver microsomal enzymes, including CYP-enzymes, were observed in rats after 45 days of aspartame administration in the diet (40 and 4000 mg/kg bw/day), but these effects were normalised after 90 days. In addition, the observed effects were mainly noted at the high dose level group, which is an extremely high dose. From the results the authors concluded that consumption of aspartame does not substantially alter the function of microsomal liver enzymes. The author's conclusion appears scientifically sound.

Vences-Meija et al. (2006) studied subchronic exposure to aspartame in rats administered aspartame by oral gavage (75 and 125 mg/kg bw/day) for 30 days. The doses used in the study do not appear to be totally irrelevant to the human exposure situation. The authors measured 7 enzymes (variants of CYP 1, 2 and 3), enzyme activities and the corresponding protein levels of the enzymes in microsome preparations from the liver and the brain. They showed that induction of CYP immunoreactive proteins by aspartame paralleled that of increased enzyme activities. Several enzymes of the different CYP families were increased (i.e. 1, 2 and 3) in the brain but not in the liver, both in terms of protein content and enzyme activity, which is noteworthy. In addition, some CYP-proteins were undetectable in the brains of control animals whereas high levels were found after aspartame treatment. Thus, the most relevant and interesting finding was that the effect was brain-specific and no enzyme induction was detected in the liver.

The paper of Vences-Meija et al. (2006) was reviewed by Magnuson et al. (2007) who stated that as pooled samples were used for microsome preparations, it was therefore not possible to assess whether the differences between groups were greater than the differences within the groups. It is not clear from the "Materials and Methods" section in the paper how the microsome preparations were analysed, i.e. how many individuals that were included in each pooled sample. Moreover, it is not clear how the statistics were calculated since the numbers of pooled and individual samples were not clearly stated. Some of the criticisms are justified, but the Organising Team does not consider this justifies excluding

the study from further evaluation. For example, the use of pooled samples may be justified when it is not practical to obtain sufficient microsomes from tissues such as the brain. Further statistical analysis may be necessary to confirm the findings of this study.

In drug development there is a great focus on CYP enzymes in different organs, including the brain, and several high quality reviews and studies have during recent years been published in this area. A tissue specific induction of several different CYP enzymes in the brain may be mediated by activation of transcription factors (Miksys and Tyndale 2008). In addition, CYP-enzymes in the brain appear to be regulated differently to those in the liver (Meyer et al. 2007; Miksys and Tyndale 2008). Consequently, the identified limitation in the experimental design of the study by Vences-Meija et al. (2006) is that pooled tissue samples were used. The view of the Organising Team is that this does not justify exclusion of the findings from a safety evaluation of aspartame. Moreover, as noted in section 1 (brain function), aspartame or its metabolites may also affect enzyme activities in the brain, e.g. acetylcholinesterase (Simintzi et al. 2007a, 2007b).Additional data on the experimental design and handling of data were requested from Vences-Meija et al. (2006) but to date no further information has been provided.

Concern has been raised that metabolites of aspartame (aspartic acid, phenylalanine, and methanol) could change the normal blood levels of these and other substances in a way that it could affect the general metabolism and physiological functions. Moreover, it is not known fully to what extent aspartame can be absorbed intact from the gastrointestinal tract and the possible consequences thereof.

The available reports mainly used high doses, and are focused on plasma changes in aspartic acid, phenylalanine and methanol (Magnusson et al. 2007). Nevertheless, the studies clearly show that these high intakes of aspartame could result in increased blood levels of phenylalanine and methanol, but changes in aspartic acid are more variable and less defined. However, there are no studies in individuals reporting sensitivity for developing adverse reactions after ingestion of aspartame at normal consumer levels. Consequently, there is a need to ascertain to what extent or not there is a systemic absorption of aspartame or its breakdown products and whether these reach levels of physiological/toxicological concern.

Refined techniques to measure changes in blood metabolite pattern are well established today and generally referred to as metabolomics. Studies using a metabolomic approach generate a profile of several hundred metabolites per analyzed blood or urine sample. Using metabolomics it would be possible to finally clarify whether a normal consumer intake of aspartame changes the profile of metabolites in the blood and whether the parent compound aspartame is absorbed into the blood. Thus, high-resolution metabolomic profiling would be possible to measure actual changes in low molecular weight compounds occurring in the blood metabolite pattern, including aspartame and its metabolites. Even modest changes in nutritional and physiological balance readily result in a pattern of effects on the general metabolic homeostasis, it is anticipated that metabolomic analysis would detect even small variations in concentrations of aspartame and its metabolites, even at low intakes. Hence, molecular fingerprints, including appropriate clustering and pattern recognition, could discriminate normal consumers from those consuming aspartame. Thus, an experimental setting based on this general layout in a controlled human study might provide novel information on possible aspartame-induced effects at normal consumer levels. In addition, it would be possible to demonstrate whether there is an actual change in the metabolite pattern that could be linked to systemic and/or adverse effects. Consequently, by enrolling individuals reporting sensitivity to aspartame, it could be possible to specifically study whether changes in metabolites are related to any specifically reported symptom. An outline for standardized metabolomic analysis has been published by Lindon et al. (2005).

Conclusion

The National Experts note that there is very little new information about the effects of aspartame and its metabolites on metabolism of endogenous and exogenous compounds. It has been shown conclusively that when administered to humans at bolus doses below the ADI value (40mg/kg/bw), aspartame is broken down to its constituent molecules, methanol, phenylalanine and aspartic acid. Plasma concentrations of the metabolites were not elevated after administration of doses close to the highest 95%ile intake of aspartame reported (13.3 mg/kg in the USA). However with increasing doses (up to 200mg/kg) all three primary metabolites can be detected in the systemic circulation in a dose-dependent manner. The highest bolus dose tested in humans (200mg/kg) is five times the ADI value. Up to date analytical methods, for example metabolomics, would enable changes in endogenous metabolic fingerprints and aspartame metabolism to be determined with more precision.

The National Experts considered that research investigating whether aspartame and its metabolites affect gene expression, protein synthesis and enzyme activities of Cytochrome P450 enzymes in the brain could be useful to extend knowledge in this area. While use of novel techniques such as metabolomics have not been considered in previous evaluations of aspartame, as they were not available at the time, it is recognised that such research is at the forefront of toxicological science and the results of such work may usefully increase the evidence base.

6. CARCINOGENICITY (INCLUDING CANCER EPIDEMIOLOGY) AND GENOTOXICITY

6.1 Carcinogenicity

Cancer epidemiology

Published epidemiological studies on aspartame since 2002 have been evaluated in this section. From 2002 on, three studies investigated the association between aspartame intake and cancer. An additional study from 2001 (Hardell et al.) was reviewed because this was not included by the SCF in 2002. Two review papers were published by Weihrauch & Diehl (2004) and Magnuson et al. (2007). The table below shows the most important results from the studies identified.

6.1.1 Summary Table: Epidemiology

Study population	Design	Dietary measure	Cancer Sites	Sample size	OR/RR**	Reference
Mothers of cases with medulloblastoma 0-5y	Case-controlled study investigated a possible association between maternal diet during pregnancy and the occurrence of brain tumours in children and more specifically medulloblastoma. Diet soda intake was one of the food categories studied	FFQ# diet soda	Study does not support an association between maternal aspartame consumption and childhood brain tumours. Intake of diet soda studied, but not aspartame per se, though the authors note aspartame was the most commonly used sweetener at time of study. Relies on mother's ability to recall consumption during pregnancy	315 children with medulloblastoma	Periconception: 1.3 (0.8-2.4) midpregnancy 1.3 (0.7-2.5)	Bunin et al. 2005
population >55y	CC	FFQ usual diet 2 years before diagnosis	Oral cavity & Pharynx, oesophagus colon rectum larynx	598 cases 304 1225 728 460 2569	0.77 (0.39-1.53) 0.77 (0.34-1.75) 0.90 (0.70-1.16) 0.71 (0.50-1.02) 1.62 (0.84-3.14)	Gallus et al. 2007

				breast	1031	0.80 (0.65-0.97)	
				ovary	1294	0.75 (0.56-1.00)	
				prostate	767	1.23 (0.86-1.76)	
				kidney		1.03 (0.73-1.46)	
					7028 controls		
Patients with malignant brain tumour	CC[*] Designed to investigate brain tumours and exposure to ionizing radiation and cellular phones	Recall consumption of low calorie soft drinks	Brain tumours	30 cases 45 controls	1.7 (0.84-3.44)	Hardell et al. Prev. (2001)
473984 Subjects 50-71y (retired persons cohort)	Pros cohort	FFQ baseline 1995	Haematopoietic and brain tumours	2106 Haematopoietic cases and 376 brain	0.98 (0.76-1.27) 0.73 (0.46-1.15)	Lim et al. 2006

[*] CC = case-cohort

[#] FFQ = food frequency questionnaire

** OR = Odds Ratio, RR = Relative Risk

Discussion

Since 2002 four epidemiology studies investigating associations between aspartame intake and cancer risk were identified: three were case-control studies and one was a prospective cohort study. These are summarised below. In addition, Magnuson et al. (2007) and Weihrauch & Diehl (2004) reviewed strengths and limitations of the studies and concluded that there is no evidence that aspartame poses a carcinogenic risk.

The study of Hardell et al. (2001) was primarily designed to investigate brain tumour occurrence from ionizing radiation and the use of cellular phones. Exposure to different agents was assessed, one of which was the intake of the artificial sweetener aspartame. Information about the consumption of low-calorie drinks was ascertained, including years of intake, times per day or week and the amount of drink consumed each time. Other aspartame containing foods were not included in the questionnaire. The publication, unfortunately, provides no data on levels of aspartame intake.

In the study of Bunin et al. (2005), mothers of children with medulloblastoma and matched controls were interviewed retrospectively about their diet during pregnancy. There was a significant association (OR 1.6, CI: 1.0-2.3, p trend=0.03) observed with frequent consumption of diet soda (≥ 2 diet soda/day) in the periconception period, in relation to medulloblastoma in children aged between 0 and 6 years. This was not statistically significant after adjustment for confounders (OR 1.3, CI: 0.8-2.4, p trend=0.35). Confounders included income level, mother's race, age for child, date of interview, gained weight as a result of nausea/vomiting, number of cigarettes /day, and total calorie intake.

The study by Lim et al. (2006) did not include foods that contained aspartame other than beverages: this was justified by the authors because at the start of the study (1995/1996) aspartame use was

limited to beverages. Addition of aspartame to coffee or tea was included to estimate daily aspartame intake. The use of aspartame containing beverages was limited for the study population, which was aged 50-71 years. Another limitation mentioned by the authors is that most participants consumed 2-3 mg aspartame/kg bw per day with limited number of participants in high intake categories. They did not detect any increase in risk estimates in the high intake categories. To calculate exposure, composition data from the Nutrition Data System for Research (University of Minnesota) and the literature were used. It is not clear whether these data are realistic for the beverages used. A general limitation of case-control studies is that they may suffer from recall and selection bias, which is not the case for a prospective cohort study as performed by Lim et al. The strength of the prospective cohort study as indicated by Lim et al. (2006) is its large size, which makes it possible to examine rare subtypes of cancer. Additionally the authors suggest that the use of histologically confirmed cases is also a strength of the study. They state that this was the first investigation of aspartame and haematopoietic cancers in humans. The findings of no increased risk of brain cancer, although limited to malignant gliomas, is consistent with animal studies and human case-control studies on brain cancer and aspartame.

Gallus et al. (2007) concluded that there was no increased risk of cancer associated with the consumption of artificial sweeteners, whilst acknowledging that one limitation of their study is that the Italian population generally has a low frequency of sweetener consumption. Additionally there was no information collected on the use of aspartame-containing soft drinks. The Food Frequency Questionnaire asked only for weekly consumption of saccharin and other sweeteners in sachets or tablets per day, so there is no specific information on aspartame.

Conclusion

In the three case-control studies and one prospective cohort study considered by the Organising Team, there is no evidence to date to support an association between aspartame and brain, or haematopoietic, or other tumour prevalence. One of the weaknesses of the studies is the estimation of aspartame intake. Whilst the National Experts do not suggest that any further studies should be undertaken, should a future study be performed, it is considered that aspartame intake should be taken into account.

Experimental Carcinogenicity

In 2002 the SCF concluded that, "taking into account all the studies that have been conducted, the frequency of spontaneous tumours in laboratory rats, the types of tumours observed and the absence of a dose-response relationship, aspartame had no carcinogenic potential on the brain in experimental animals".

Published experimental carcinogenicity studies on aspartame since 2002 have been evaluated in this section, and the table below shows the most important results from the studies identified.

6.1.2 Summary Table: Carcinogenicity

Study Title	Summary of details / specifics of study	Comments on significance
Toxicology studies of aspartame (CAS No. 22839-47-0) in genetically modified (FVB Tg.AC hemizygous) and B6.129-Cdkn2atm1Rdp (N2) deficient mice and carcinogenicity studies of aspartame in genetically modified [B6.129-*Trp53*tm1Brd	Groups of p53 haploinsufficient, Cdkn2a deficient mice and control animals were fed diets containing up to 5% aspartame for 9 months. Exposure to aspartame had no effect on survival in any of the animal groups. No increases in tumours were seen in males or	The mouse models used are specially bred to be highly sensitive to cancer-causing agents. The authors note that because at the time of the study the p53 haploinsufficient mouse was a new model, there was uncertainty whether the model possessed

(N5) haploinsufficient] mice NTP (2005)	females of either strain.	sufficient sensitivity to detect a carcinogenic effect.
Aspartame induces lymphomas and leukaemias in rats Soffritti et al. (2005)	Lifetime carcinogenicity study in rats administering aspartame in diet at concentrations of 100,000, 50,000, 10,000, 2,000, 400, 80, or 0 ppm. Authors report an increased incidence of malignant-tumour–bearing animals with a positive significant trend in males and in females, in particular those females treated at 50,000 ppm. An increase in lymphomas and leukemias, a statistically significant increased incidence of transitional cell carcinomas of the renal pelvis and ureter and their precursors (dysplasias) in females and an increased incidence of malignant schwannomas of peripheral nerves in males.	The study is flawed. Protocol is not standard as required for two year carcinogenicity studies - so see increased age related tumour rate (especially as survival of animals in control group was poorer than treated rats). High rate of infection rate in the animals. Housing does not meet GLP, animals housed in different rooms, too many animals per cage, not randomised; no information on diet - stability of aspartame is not characterised, or homogeneity stated. At 10% dose level no compensation for nutritional imbalance, food consumption decreased as aspartame concentrations increased. Decreased body weight (no data are given, only graph). Data regarding haematology are incomplete.
First Experimental Demonstration of the Multipotential Carcinogenic Effects of Aspartame Administered in the Feed to Sprague-Dawley Rats Soffritti et al. Env. Health Perspect. (2006)	Lifetime carcinogenicity study in rats administering aspartame in diet at concentrations of 2,000, 400, or 0 ppm. Authors report a significant dose-related increase of malignant tumour–bearing animals amongst males, particularly in the group treated with 2,000 ppm aspartame. A significant increase in incidence of lymphomas/leukemias in males treated with 2,000 ppm and a significant dose-related increase in incidence of lymphomas/leukemias in females particularly in the 2,000-ppm group and a significant dose-related increase in incidence of mammary cancer in females particularly in the 2,000-ppm group.	Flawed, Soffritti (2005) reports on same study. Also 2005 paper reports statistically significant increase in lymphomas and leukemias in females whilst this study reports identical incidences but no statistically significant increase in males & females.
Results of long-term carcinogenicity bioassay on Sprague Dawley rats exposed to aspartame administered in food Belpoggi, Soffritti et al. Ann N. Y. Acad Sci. (2006)	Lifetime carcinogenicity study in rats administering aspartame in diet at concentrations of 100,000, 50,000, 10,000, 2,000, 400, 80, or 0 ppm. The authors report an increased incidence of malignant tumour-bearing animals, with a positive significant trend in both	Flawed, Soffritti (2005) reports on same study.

	sexes, and in particular in females treated at 50,000 ppm compared to controls; an increase in lymphomas-leukemias, with a positive significant trend in both sexes, and in particular in females treated at doses of 400 ppm and above. A statistically significant increased incidence of transitional cell carcinomas of the renal pelvis and ureter in females and an increased incidence of malignant schwannomas of the peripheral nerves in males.	

Discussion

The studies by Soffritti et al. (2005, 2006), as reported, suffer too many methodological flaws to be taken into consideration when determining the carcinogenic potential of aspartame. These studies are not considered valid.

In addition to examining the conduct of the study, the interpretation of the data leading to the results reported by Soffritti et al. (2005, 2006) have been re-evaluated (EFSA, 2006). This re-evaluation was based on information provided in the publication by the Ramazzini Foundation, unpublished reports, and other existing scientific literature. It also included the results of unpublished transgenic mice studies conducted on aspartame by NTP and an unpublished epidemiological study of aspartame by the National Cancer Institute.

The updated opinion published by EFSA's ANS Panel in March 2009 (EFSA, 2009) on the new Ramazzini Foundation study on life-span exposure to low doses of aspartame in rats (Soffritti et al, 2007) and taking into consideration data submitted by the Ramazzini Foundation in February 2009 was also noted by the Organising Team. The Panel concluded that on the basis of all the evidence currently available, there is no indication of any genotoxic or carcinogenic potential of aspartame and no reason to revise the previously established Acceptable Daily Intake for aspartame of 40 mg/kg body weight.

The carcinogenic potential of aspartame has also been considered by the VKM. VKM considered the studies of Soffritti et al. noting from the EFSA Opinion (2006):

1. The increased incidence of lymphomas/leukaemias reported in the study was unrelated to the treatment with aspartame given the high background incidence of chronic inflammation and; the lack of positive dose-response relationship.

2. Concerning the malignant schwannomas, the numbers of tumours were low, the dose-response relationship was very flat over a wide dose range and there remained uncertainties regarding the diagnosis of such tumours.

3. The preneoplastic and neoplastic lesions of the renal pelvis, ureter and bladder along with renal calcification were probably treatment-related However, such effects are specific to rats and caused by high dosages of irritant chemicals and have no relevance for humans.

4. The aggregation of all malignant tumour incidences or all malignant tumour-bearing animals for statistical purposes is not scientifically justified.

In conclusion the VKM agreed with the EFSA evaluation of these studies (2006) and noted that the incidence of lymphomas/leukemias was not very high. Moreover the incidence in the control group and the highest aspartame dosage groups fell within the range of historical control values. The inclusion of both hyperplasic and dysplastic lesions, and carcinomas in the aggregation of all malignant tumours is not supported by VKM.

Conclusion

Regarding experimental carcinogenicity, there is no new evidence published since 2002 that conflicts with the SCF Opinion (2002) on aspartame and therefore the National Experts conclude that there is no evidence to suggest that aspartame is carcinogenic.

Given the totality of evidence considered in all of these reviews there is no need for further scientific investigation into the carcinogenicity of aspartame.

6.2 Genotoxicity

Based on the experimental studies available at the time, the SCF concluded in 2002 (SCF, 2002) that aspartame is not mutagenic and does not have clastogenic potential hence it was not genotoxic. Three articles relating to the potential genotoxic/mutagenic effects of sweeteners and their metabolites published since 2002 have been identified for review, two of them resulting from the same research team (Rencuzogullari et al. 2004, Canimoglu et al. 2006). The third study focuses on the genotoxic effect of the food sweetener maltitol (Canimoglu et al. 2006) and therefore this article was not considered further. Just two studies focus on genotoxicity and mutagenicity of aspartame (Rencuzogullari et al. 2004 and Bandyopadhyay et al. 2008). No other publications after 2002 report the potential genotoxic/mutagenic effects of aspartame metabolites.

6.2.1 Summary Table: Genotoxicity

Study Title	Summary of details/ specifics of study	Comments on significance
Genotoxicity of Aspartame Rencuzogullari E. et al. Drug and Chemical Toxicology (2004)	4 *in vitro* assays were used to evaluate genotoxicity and mutagenicity of aspartame: chromosome abberrations (CA) test, chromatid exchange (CE) test and micronucleus test on human lymphocytes isolated from 4 different donors (2 males, 2 females). Mutagenicity of aspartame was investigated by Ames/ Salmonella/ microsome test on two bacterial strains *Salmonella typhimurium* TA98 (frameshift mutation) and TA100 in the presence and absence of S9 mix.	There is a great impact of statistical analysis: cells were isolated from 4 different donors (2 males, 2 females) and results are presented as if one cell model has been used and individual results are not presented; the number of examined cells is too low in all used tests. There was no dose-dependent effect.
Genotoxicity Testing of	The mutagenicity of the three low-	Aspartame did not induce mutagenic

Low-Calorie Sweeteners: Aspartame, Acesulfame-K and Saccharine Bandyopadhyay A. et al. Drug and Chemical Toxicology (2008)	calorie sweeteners was tested on *Salmonella typhimurium* TA97a (frameshift mutation) and TA100 (base-pair substitution) strains in the Ames/Salmonella/microsome test in the absence and presence of the S9 mix at different concentrations of sweeteners.	effects in both strains of *Salmonella typhimurium* in the absence or in the presence of S9 mix.
	The genotoxic potential of sweeteners was tested by comet assay in the bone marrow cells isolated from Swiss albino mice *Mus musculus* after oral administration with different concentrations of three sweeteners (ASP; 7, 14, 28 and 35 mg/kg body weight), (ASK; 150, 300 and 600 mg/kg body weight), (saccharin; 50, 100 and 200 mg/kg body weight).	Although statistically, aspartame significantly induced DNA damage at the highest tested concentration in comparison with control cells, there are limitations in statistical analyses and the number of analyzed cells is too low (only 50 cells compared to the standard procedure of analyzing 500 cells) which affects the statistical validity of the results.

Discussion

In the first study of Rencuzogullari et al. (2004) the authors use 4 *in vitro* assays on human lymphocytes for genotoxicity and 1 *in vitro* assay on *Salmonella typhimurium* TA 98 and TA 100 in the presence and in the absence of S9 mix to detect mutagenicity. The tested concentrations of aspartame did not induce mutagenic effects in either strain of *Salmonella typhimurium* in the absence or presence of S9 mix and hence there was no dose-dependent effect noted. Aspartame did not induce chromatid exchange. Aspartame induced a significant increase of chromosome aberrations (CAs) at all concentrations, however this effect was not dose-dependent. Micronucleus formation was induced by the highest tested concentration at after 24 and 48 hours of treatment. The selection of the tested concentrations of aspartame used is not clear. Furthermore, any supplementary information relevant to cytotoxicity was not presented, which could affect results of CA test and micronucleus assay. The low number of analyzed cells has a significant impact on the statistics and combined with a lack of clear description of some methods and data interpretations makes interpretation of the results unreliable. No conclusion could therefore be derived from this study.

In the second study of Bandyopadhyay et al. (2008) the genotoxic potential of sweeteners was tested by comet assay in the bone marrow cells isolated from Swiss albino mice *Mus musculus* after oral administration with different concentrations of aspartame and the mutagenicity was tested on *Salmonella typhimurium* TA97a and TA100 strains in the Ames/Salmonella/microsome test in the absence and presence of the S9 mix at different concentrations of sweeteners. Aspartame did not induce mutagenic effects in both strains of *Salmonella typhimurium* in the presence or absence of S9 mix.

In the same study, DNA damage was significantly induced at the highest tested concentration of

aspartame in comparison with control cells. The number of analyzed cells was however too low which has an impact on the statistical relevance of the outcome.

Genetic toxicology of aspartame was also studied by NTP in 2005. Mutagenicity and genotoxicity were tested in Salmonella typhimurium, rat bone marrow cells and mouse peripheral blood erythrocytes.

No mutagenicity was detected in strains TA98, TA100 and TA1535 with and without metabolic activation.

An acute bone marrow micronucleus test was conducted with aspartame administered by gavage to male F344/N rats. No increase in micronucleated polychromatic erythrocytes was observed at any dose level.

Peripheral blood micronucleus tests were conducted after 9 months exposure of Tg.AC hemizygous mice, p53 haploinsuffucient and Cdkn2a deficient mice fed with aspartame dosed feed. Negative results were obtained in male and female Tg.AC hemizygous and Cdkn2a deficient mice. Negative results were also obtained with male p53 haploinsufficient mice. In female p53 haploinsufficient mice, the results of the micronucleus test were judged to be positive, based on a significant trend test and the increased frequency of micronucleated erythrocytes seen in the 50,000 ppm group. Although the increase was statistically significant, the response was small of approximately one additional micronuclei per one thousand cells. For all three strains of mice, the percentage of polychromatic erythrocytes (PCEs) was not significantly altered by treatment with aspartame.

Conclusion

Neither the paper of Bandyopadhyay et al. (2008) nor that of Rencuzogullari et al. (2004) nor the NTP studies (2005) provide additional evidence that aspartame presents a mutagenic risk. With respect to genotoxicity, the study of Bandyopadhyay et al. (2008) showed possible DNA damage at the highest dose of 35 mg aspartame /kg bw but also has statistical limitations. In the NTP (2005) study only female p53 haploinsuffucient mice showed statistically significant increase in the frequency of micronucleated normal chromatic erythrocytes at the highest concentration. This response, however, was small, resulting in approximately one additional micronuclei per 1 000 cells. The National Experts conclude that there is no evidence to indicate that aspartame has genotoxic potential.

7. Review of Anecdotal Data

Ongoing public concern with respect to the risks of aspartame use in food is acknowledged to be due in part to anecdotal case history reports by individuals who have attributed various symptoms and illnesses to aspartame consumption. This section of the report addresses the anecdotal data that was available to EFSA and the Organising Team.

The objectives of undertaking the work on this information were to:

1. determine the scope of the symptoms reported
2. document the information on symptoms in a database in order to carry out analysis on the information
3. determine whether a dose response effect of aspartame can be established
4. data source

The source of data used comprised of cases compiled by Dr. H.J. Roberts in his book 'Aspartame Disease an Ignored Epidemic', (Roberts, 2001) and 68 cases documented in 23 case reports published in the peer reviewed scientific literature (Eshel and Sarova-Pinhar, 1993; Newman and Lipton, 2001; Smith et al. 2001; Robbins, 1999; Blumenthal and Vance, 1997; Gerrard, Richardson and Donat, 1993; Fernstrom, 2008; Jacob and Stechschulte, 2008; Mortelmans et al. 2008; Hill and Belsito, 2003; Camfield et al. 1991; Reed at al. 1993; Gulya et al. 1992; Tollefson et al. 1992; Geha, 1992; McCauliffe and Poitras, 1992; Drake, 1986; Johns, 1986; Walton, 1986; Bradstock et al. 1986; Kulczycki, 1986; Wurtman, 1985; Novick, 1985). These cases reported in the peer-reviewed literature, like those in Dr. Roberts' book, were self-reported.

Three scientific literature publications from Roberts, (Roberts, 1991,1997, and 2008) were not included in this data base to reduce the risk of duplication of the same cases documented in Roberts, 2001.

The total number of cases entered was 1, 135.

In addition, there were 81 cases submitted directly to the Organising Team by interest groups as a result of the call for data. These cases were not available for coding at the time the database was created and were therefore considered separately.

Data Handling

In order to create a data base of the case histories, all identified cases were given a unique coding tag to facilitate easy reference to the original source. For each case, information was recorded on: whether the case had been peer reviewed or not; gender; nationality; age; the source of the product containing aspartame that was reported to trigger an adverse reaction to aspartame; the duration of exposure; the dose of aspartame mg/day; pre-existing symptoms; number of symptoms reported; duration of symptoms; whether the symptoms were independently verified; the time to develop symptoms after the aspartame challenge; the period of withdrawal; time to stop symptoms after withdrawal; symptoms reported after removing aspartame exposure; whether there was an aspartame rechallenge; if so the time for symptoms to reappear; the duration of exposure during the rechallenge; symptoms reported after rechallenge; time for the symptoms to stop after rechallenge; allergies reported to have developed. All symptoms were classified according to the internationally agreed WHO ICD10 codes available from http://www.who.int/classifications/apps/icd/icd10online/ (2007 version) to facilitate comparison between this data and other data sets.

A note was made of any potential confounding factors, and other information that might be relevant.

The Organising Team noted that for most of the case history reports many of these details were not given.

All data were entered into a formatted Excel spreadsheet, which was then transferred to SAS (SAS Enterprise Guide 4.1) to formulate the data base for analysis purposes. To ensure quality control, data entries and symptom coding were randomly checked and verified by a second team member with further systematic checks for duplicate cases and symptoms by a third team member when cleaning the SAS database prior to data analyses.

Where possible (i.e. where it was reported), dietary exposure estimation was undertaken. Details of the methods employed for aspartame dose estimation are given in Appendix 2.

Results

The total number of cases considered was 1135. The number of cases where symptoms were classified was 1,059. No symptoms were recorded for 76 cases as the information provided was not specific enough to allow classification.

Of the total number of reports from the book of Dr Roberts (Roberts, 2001) entered in the data base, 689 were female, 299 were male and 77 were unclassified (the gender was not specified). Forty three cases were children and an additional 154 cases were documented with the index case (i.e. friends and relatives).

The total number of symptoms reported from all sources was 4,281, as most cases reported more than one symptom.

The top 20 symptoms reported in terms of frequency and percentage are presented in Data Table 1. Headache (28.5%), dizziness and giddiness (19.2%) and mild cognitive disorders (17.4%) were the top three symptoms reported. Data Table 1 shows the top 20 symptoms as number of cases and percentage.

Data Table 1. The top 20 symptoms reported for 1059 aggregated case study data.

Symptom	Number of cases	Percentage of total cases[2]
Headache	302	28.5
Dizziness and giddiness	204	19.2
Mild cognitive disorder	184	17.4
Recurrent depressive disorder	153	14.4
Epilepsy	97	9.2
Other symptoms & signs involving cognitive function and awareness	94	8.9
Pain in joint	92	8.7
Irritability and anger	87	8.2
Panic disorder [episodic paroxysmal anxiety]	84	7.9
Nausea and vomiting	80	7.5

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[2] For example in the case of headache, there were 302 reports out of 1059 cases, giving a percentage of 28.5% of cases reporting headache symptoms.

Nonorganic insomnia	78	7.3
Malaise and fatigue	72	6.8
Palpitations	69	6.5
Speech disturbances, not elsewhere classified	66	6.2
Functional diarrhea	64	6.0
Abdominal and pelvic pain	63	5.9
Tinnitus	63	5.9
Blindness and low vision	55	5.2
Irritant contact dermatitis	53	5.0
Somnolence, stupor and coma	52	4.9

The examination of the additional anecdotal reports received by EFSA from 'Mission Possible' in the UK and France, shows that out of 81 cases the most frequently reported primary symptoms (70%) relate to conditions of the eyes (as coded H54.2, H54.5 and H57.1 in the WHO ICD10 coding system used, which relates to low vision in one or both eyes and ocular pain respectively as defined by the WHO).

Data Table 2 shows the most common symptoms reported in conjunction with headache (302 cases reported). The most common symptoms were dizziness and giddiness (35.1% or 106) mild cognitive disorder (26.8% or 81 cases) and recurrent depressive disorder (20.9% or 63 cases).

Data Table 2: Symptoms most frequently reported (greater than 10%) with headache (302 cases).

Symptom	Number of cases	Percentage[3]
Dizziness and giddiness	106	35.1
Mild cognitive disorder	81	26.8
Recurrent depressive disorder	63	20.9
Irritability and anger	49	16.2
Nausea and vomiting	43	14.2
Pain in joint	42	13.9
Panic disorder [episodic paroxysmal anxiety]	41	13.6
Other symptoms & signs involving cognitive function and awareness	40	13.2
Palpitations	38	12.6
Abdominal and pelvic pain	33	10.9
Malaise and fatigue	33	10.9
Speech disturbances, not elsewhere classified	32	10.6

Only 328 out of 1059 cases provided any information on types of products consumed. Where provisional estimations were made (with assumptions being from the unspecific information provided, based on maximum possible intake see Appendix 2) for the products consumed (e.g. diet drinks, gelatine desserts, yogurts) the mean dietary intake was estimated to be 1050 mg/day with a range between 4 mg/day and 10560mg/day.

Of all 1135 cases, 710 cases reported one or more specific sources of aspartame that possibly caused their symptoms. One person reported eight foods. Most often one food was named; in 208 cases two foods were reported. Data Table 3 describes the number of times a food or food group was mentioned. Diet soft drinks was identified 308 times.

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[3] For example, Dizziness reported with 106 cases of Headache of a total of 302 cases of Headache

Data Table 3: Food or food group self attributed to be responsible for adverse aspartame effects.

Type of food	Number of times mentioned
Diet soft drink	308
Diet coke	244
Table top sweeteners	135
Gum, sweets	73
Sweetened coffee/tea	61
Desserts	33
Medicines, nutritional supplements	22
Chocolate mixes	17
Cereals	14
Other	121

Discussion

The objectives of considering the anecdotal case information were to determine the scope of the symptoms reported; to document the information in a database that may allow an analysis on that information for the reported cases and to determine whether a dose-response effect can be established for the observed symptoms. The National Experts believe that this is the first time a group of experts has undertaken such an assessment of anecdotal reports on aspartame.

The National Experts note that the data on case information was gathered from self reporting individuals and as such the details available for each reported case varied greatly and the data does not provide a robust initial evidence base as independent validation for each was not available. For most cases there was not sufficient information to identify any estimate of dietary intake and it has not been possible to establish aspartame as causing the symptoms reported and in relation to dose-response, it was not possible to come to a meaningful conclusion as relatively few cases reported dose and of the cases that did report dose and/or duration the detail reported was highly variable.

It has been possible to identify the most frequently reported symptoms with the most frequent being headache, dizziness and cognitive/depressive disorders.

Acknowledging the limitations in the anecdotal data and the caution that must therefore be exercised in interpreting any analysis carried out, the National Experts consider that the information gathered can be useful in guiding the design and format of any investigative study that may be undertaken to determine individual sensitivity to aspartame. How or what the individuals are sensitive to and the underlying mechanism of that sensitivity to aspartame can also be explored in relation to the symptoms that are documented and collated in this report.

The National Experts recommend however that where such case information is being gathered, for any analysis to be of benefit the information must be gathered in a detailed and consistent manner such as the methods employed for general descriptive studies (Hennekens et al, 1987; Vandenbroucke, 2001; Glasziou et al. 2004; Sargeant et al, 2005).

Conclusions

The National Experts conclude that based on analysis of the database of case histories, there are a number of symptoms that are recurrently reported by individuals who believe that they are caused by

aspartame ingestions. These include headache, dizziness and cognitive/depressive disorders. The National Experts consider that the information gathered in this analysis can be useful in guiding the design and format of any investigative study that may be undertaken to determine individual sensitivity to aspartame.

CONCLUSIONS AND RECOMMENDATIONS

This report has described the outcome of an initiative facilitated by EFSA at the request of its AF, to identify new publications on aspartame to identify possible areas of discrepancies, or knowledge gaps, in the body of evidence on aspartame safety since the SCF evaluation of 2002, and to consider options to address these discrepancies and/or gaps, if any. All documents identified were reviewed and considered in the areas of exposure data, and possible effects on brain function, satiation and appetite, allergenicity and immunotoxicity, metabolism of endogenous and exogenous compounds, genotoxicity and carcinogenicity (including cancer epidemiology).

The Organising Team also considered the available, but non peer reviewed, 'case histories' which largely consisted of anecdotal reports by individuals who attributed various symptoms and illnesses, including headaches, seizures, memory loss, vision/eye conditions, allergies and gastro-intestinal symptoms directly to aspartame consumption.

The SCF concluded in 2002 that high-level consumers, both adults and children, were unlikely to exceed the ADI of 40 mg/kg bw per day for aspartame. Consumption by subgroups such as diabetics that are likely to be high consumers of foods containing aspartame were also well below the ADI. From the available data the National Experts conclude that no population group is likely to exceed the ADI for aspartame on a regular basis, and the data on aspartame exposure since 2001 confirm the SCF conclusions of 2002.

The SCF Opinion of 2002 had in particular considered possible neurological effects of aspartame, in the light of new reports (up to 2002) on the consumption of aspartame in relation to the onset of brain tumours and seizures, headaches, allergies, and changes in behaviour, mood and cognitive function. In relation to this area the National Experts conclude that there is still no substantive evidence that aspartame can induce such adverse effects, as earlier concluded by the SCF. Several *in vitro* and *in vivo* studies indicate that aspartame or its metabolites may affect certain enzyme activities in the brain including acetylcholinesterase Na^+/K^+ -ATPase or CYP enzymes. The National Experts consider that the biological relevance of such finding is not clear, particularly the relevance of findings in *in vitro* studies in which the toxicokinetic and toxicodynamic behavior of aspartame *in vivo* is not fully reflected. The National Experts consider however that the scientific literature needs to be monitored for further research and mechanistic explanations related to this area.

With regard to satiation and appetite, the National Experts note that it has been suggested that aspartame may have modulating effects on these responses, as the experience of sweet taste without a calorific intake has an influence on appetite for both sweet and savoury foods. The National Experts conclude that there is little or no substantive data suggesting that aspartame affects appetite/hunger, food intake. A study focusing on aspartame, such as that performed by Just et al. (2008) which looked at cephalic insulin response in healthy fasting volunteers after taste stimulation, comparing sucrose, starch and saccharin, may warrant further consideration. However at this point in time such considerations do not form the basis for recommending a re-evaluation of the safety of aspartame Although anecdotal reports in the early 1980s suggested that aspartame might be associated with allergic-type reactions, several clinical studies have shown that when the allergic-type reactions raised in these case reports were evaluated under controlled conditions aspartame is no more likely to cause reactions than placebo. The weight of evidence collected demonstrates that it is not likely that aspartame is associated with allergic-type reactions in experimental models or humans.

In some recent case reports associations have been made between aspartame intake, in particular the subsequent exposure to the aspartame metabolite formaldehyde, and Type IV Delayed Type Hypersensitivity (DTH) reactions in patients with proven contact sensitization to formaldehyde. However, to confirm the associations observed in these two case studies with only seven patients,

larger studies would be needed involving double-blind placebo-controlled challenges with aspartame and placebo exposures and the inclusion of well defined control groups.

The National Experts further conclude that no new evidence was found to indicate that aspartame or any of its metabolites is immunotoxic.

The National Experts note that there is very little new information about the effects of aspartame and its metabolites on metabolism of endogenous and exogenous compounds. It has been shown conclusively that when administered to humans at doses close to or below the ADI value of 40mg/kg/bw, aspartame is broken down completely. However with increasing doses the metabolites can be detected in the systemic circulation. Up to date analytical methods, for example metabolomics, would enable changes in endogenous metabolic fingerprints and aspartame metabolism to be determined with more precision. The National Experts considered that research into whether aspartame and its metabolites affect gene expression, protein synthesis and enzyme activities of different Cytochrome P450 enzymes in the brain could be useful to extend knowledge in this area. While the use of novel techniques such as metabolomics has not been considered in previous evaluations of aspartame, as they were not available at the time, it is recognised that such research is at the forefront of toxicological science.

In considering the endpoint of carcinogenicity including cancer epidemiology, the Organising Team noted that the three case-control studies and one prospective cohort study considered provided no evidence to date to support an association between aspartame and brain, or haematopoietic, or other tumour development. However the National Experts note that existing epidemiological data does not include accurate estimates of aspartame intake. The National Experts consider that this aspect should be taken into account in planning any future studies.

The National Experts conclude that there is no new evidence on carcinogenicity in animals published since 2002 that conflicts with the SCF Opinion (2002) on aspartame and the EFSA Opinion of 2006. There is no specific need for further investigation on carcinogenicity.

There is also no new evidence that requires a revision of the existing opinions indicating a lack of genotoxic/mutagenic potential of aspartame.

On the anecdotal evidence, the National Experts conclude that based on analysis of the database of case histories, there are a number of symptoms that are recurrently reported by individuals who believe that they are caused by aspartame ingestion. These include headache, dizziness and cognitive/depressive disorders. The National Experts consider that the information gathered in this analysis can be useful in guiding the design and format of any investigative study that may be undertaken to determine individual sensitivity to aspartame.

Overall, the National Experts did not identify any major gaps in information that in their opinion require addressing urgently. However, several suggestions are made within the report for additional studies which would add to the available knowledge on aspartame and its metabolites. In conclusion, the National Experts have not identified any new evidence to recommend to EFSA that the previous Opinions of EFSA and the SCF need to be reconsidered.

The conclusions and recommendations of this report reflect those of the national experts involved in the meetings on aspartame and do not necessarily represent the views of EFSA.

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APPENDIX 1: PAPERS CONSIDERED BY THE ORGANISING TEAM BUT NOT INCLUDED IN THE REPORT

Title of Paper	Summary of Content	Accepted for Report (Yes/No)	Comments (Criteria)
CDC Summary cases	Summary of case reports up until 1998	Yes	Case information
Peer-reviewed case reports	Individual published case reports	Yes	Peer-reviewed Case Information
Roberts H J. 2001. Aspartame Disease: An Ignored Epidemic. Sunshine Sentinel Pr Inc.	Published book of case reports	Yes	Collated case information
Submitted case information - Mission Possible US, UK, FR + Mark Gold	Collated case reports	Yes	Collated Case information
Victoria Innes-Brown, 2002/2008. My Aspartame Experiment. Report from a Private Citizen. Writers Without Borders	Non-peer reviewed rat experiment	No	GLP and OECD guidelines not followed; deficiencies in methodology
Monte W. 1984 'Aspartame: Methanol and Public Health' Journal App Nut 36	Published report not considered previously	No	Review paper - No new information/studies.
Monte W. Is your Diet Sweetener killing you? Fitness Life 2006 Nov. 33: 31-33; A Deadly Experiment, Fitness Life 2007 Dec.34:38-42); Bittersweet: Aspartame Breast Cancer Link, Fitness Life 2008 Feb 34:21-22	Non-peer review published reports.	No	Unsubstantiated opinions, no new scientific evidence presented
Gold, M. 2008 Aspartame and Manufacturer Funded Scientific Reviews	Unpublished Report	No	Review/opinion paper. No new scientific evidence presented

Gold M. 2003. Independent Analysis of the Opinion of the European Commission Scientific Committee on Food: Update on Safety of Aspartame /E951	Unpublished Report	No.	Opinions supported by anecdotal evidence only. Issues raised outside scope of terms of reference.
Olney, 1994. Excitotoxins in Food. Neurotoxicity, 15, 535-544	Published report not considered previously	No.	Issues raised considered by SCF Opinion of 2002.
Gombos et al. 2007 Effect of Aspartame Administration on Oncogene and Suppressor gene Expressions. In Vivo 21, 89-92	Published report not considered previously	No	Lack of relevant data to allow interpretation
Huff et al. 2007. Aspartame Bioassay Findings Portend Human Cancer Hazards, Int J Occup Environ Health 13, 446-448	Published report not considered previously	No	No new scientific evidence presented.
Sonnewald 1995 Effects of aspartame on 45Ca influx and LDH leakage from nerve cells in culture Neuroreport. 6, 318-320	Published report not considered previously	No	Inconclusive *in vitro* study; exposure corresponding to that used in the cited study is not physiologically relevant.
Shephard 1993. 'Mutagenic activity of peptides and the artificial sweetener aspartame after nitrosation' Food Chem Toxicol. 31, 323-329	Published report not considered previously	No	The results provided by the author are inconclusive for aspartame
Swithers, 2008 'A Role for Sweet Taste: Calorie Predictive Relations in Energy Regulation by Rats' Behavioural Neuroscience 122, 161-173	New published report	No	Study does not refer to aspartame and outside scope of terms of reference
John Garst. Unpublished theory on Folic Acid depletion.	Unpublished report/comment	No	Inconclusive and contradictory cause/effect proposals.
Huff 2007 Letter to US FDA Commissioner. Int J Occ Environ Health. 449-450	Published letter	No	Opinion on Soffritti study predating EFSA Opinions
US Patent for variation of aspartame – heat stability for cooking	Patent relating to instability of aspartame in cooking	No	Outside scope of terms of reference mandate

US Health Subcommittee Hearing on integrity of scientific data submitted by Searle Co. to FDA 1976	Hearing report	No.	Outside scope of terms of reference.
Rao, McConnell, Waisman. 1972. 52 Week Oral Toxicity Study in the infant monkey	Original early study	No	Early study with no significant conclusions
Pilkington and Nunn 2002. 'Evaluation of the Dietary Additive Aspartame ('Nutrasweet') as a risk factor for central nervous system tumours', Kings College	Unpublished scientific report	Not appraised	Extensive enquiries failed to locate paper
Walton R. 1994 Correspondence in Biol. Psychiatry 36, 206-210	Published correspondence relating to 'Adverse Reactions to Aspartame: Double-Blind Challenge in Patients from a Vulnerable Population' including letter from Butchko	No	No new information provided; inconclusive study.
Walton R. 1988 The possible role of aspartame in seizure induction	Published conference paper - reference to be confirmed. Case information	Yes	Case information
Walton, R. 1988 Survey of aspartame studies: correlation of outcome and funding sources	Unpublished report	No	No new scientific evidence presented
Nordmann H, Additional Information on intake from Trinity College Dublin, 1999 and comment on paper by Walton, 1994.	Unpublished report to Nutrasweet	No	No new scientific evidence presented
Olney 1996 Increasing Brain Tumour rates - is there a link to aspartame? Journal of Neuropathology and Experimental Neurology	Paper referenced by the SCF, 2002.	No	Previously considered by SCF 2002
Millstone. 1996 Fresh Doubts about aspartame safety: how to respond?	Unpublished paper	No	Review paper. No new scientific evidence presented
Millstone. 2006 Aspartame: the litmus test for the FSA and EFSA	Unpublished paper	No	Opinion/comment on the Searle investigations and Soffritti studies. No new scientific evidence presented.

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Millstone E, Abraham J. Additives A Guide for Everyone. Penguin Books Ltd 1988	Published Book	No	No new scientific evidence presented
Erik Millstone. 1994 Sweet and Sour: the Unanswered Questions about aspartame The Ecologist, 24 71-74	Published paper	No	Review paper. No new scientific evidence presented
The following papers relate to the history of the approval of aspartame and the investigations into the practices at the Searle laboratories.			
Index of documents about G D Searle testing and approval process from Senator Howard Metzenbaum's staff, 1986	Details of documents relating to the investigations into the shortcomings of the testing and approval process for aspartame.	No	The documents provide the support for controversy regarding the regulatory process and investigations at the time aspartame was first marketed.
Establishment Inspection Investigation Report (EIR). FDA Searle Task Force, 1978	Investigation into Hazleton Laboratories, Virginia regarding studies on aspartame		
Final report: FDA's Bureau of Drugs Searle Task Force, 1976	Report on the investigations into Searle and Hazleton laboratories		No new scientific evidence is presented in them.
Memorandum for reviewing aspartame studies, Dr Adrian Gross, 1976	Memorandum regarding the arrangements of the review of the safety studies relating to aspartame		
Letter from FDA Chief Counsel to US Federal Attorney, 1977	Instruction to convene Grand Jury to investigate Searle Lab.		Concerns over the procedural and regulatory matters are outside the scope of the terms of reference to consider further.
Internal memo, US Federal Attorney 1977	Memo regarding potential conflict of interest of US Federal Attorney in Searle investigation.		
Bressler Report – FDA Report on Searle research 1977 (Parts 1 & 2)	Report on the investigation into practices at Searle laboratories, including studies on aspartame		
FDA memo from the Bureau of Foods Task Force to the Acting Director of the FDA Bureau of Foods, 1977	Internal FDA memo. summarising the findings of the Bressler report.		
Universities Associated for Research and Education in Pathology Inc. (UAREP) Report, 1977	Introduction only to the UAREP validation studies of Searle projects		
Correspondence on quality and reliability of the UAREP review between Dr Adrian Goss and	Views on the conclusions of the UAREP Report.		

Senator Metzenbaum, 1987			
Report of the Public Board of Inquiry (PBOI), 1980	PBOI inquiry into controversy concerning the safety of aspartame.		
Internal Memoranda from FDA Toxicologists, 1981	Opinions on safety in relation to the brain.		
Prof. Richard Wurtman, MIT Letter. New England Journal of Medicine 1983	Views on the potential use in soft drinks to provoke adverse neurological symptoms		
US Senate Nutrasweet Bill, 1985.			
Letter to US Senate Judiciary Committee 1986	Concerns raised about the conduct of US Federal Attorney's Office in relation to the Searle investigation		
Letter from Nutrasweet to E Millstone, 1987	Correspondence referring to three studies authenticated by FDA as not having ever been repeated.		
Newspaper article: 1987	Commentary and views on the approval and investigation process		
Senate Hearing of Committee on Labor and Human Resources United States Senate, 1987.	Hearing transcript		

APPENDIX 2: METHODOLOGY EMPLOYED FOR ASPARTAME DOSE ESTIMATION

It was not possible to determine the exact quantity of aspartame consumed by the individual in each case. While all the cases appear to have some exposure, from the information provided, this was not quantifiable. Therefore to provide some estimations, it was decided to take a precautionary approach to the data and assumed, in all instances, that the maximum permitted level of aspartame was present in the food and drink consumed and the entire portion was consumed. Data from the "maximum usable doses for ready to-eat foodstuffs" (obtained from the European Parliament and Council Directive 94/35/EC on sweeteners for use in foodstuffs as amended by directive 2006/52/EC of 5 July 2006 [11]) was used to estimate dietary exposure.

In isolation, the data derived from many case reports were not detailed enough to determine the dose. Therefore a number of initial basic general assumptions had to be made, these are listed below:

The aspartame content in foodstuffs calculated in this way were subsequently compared to those known to be used in foodstuffs reported in an Italian study (Arcella et al. 2004) and aspartame food composition data provided by Cyprus, and a high level of variability in Aspartame content was noted between products, within brands and over different years even with respect to the same branded item formulations.

- 1 Can = 330 ml
- 1 Cup = 190 ml
- 1 Glass = 250 ml
- 1 Small Bottle = 275 ml
- 1 Bottle = 330 ml
- 1 quart = 950 ml
- 1 imperial gallon = 4400 ml
- 1 bowl = 35 g bowl of breakfast cereal
- 1 serving = 100 g bowl of fat based desert
- 10 Ounce Can = 275 ml
- 12 Ounce Can = 330 ml
- 16 Ounce Can = 475 ml
- 1 stick of gum = 1.5 g
- 1 Sweetener tablet contains 20 mg of aspartame
- 1 packet of sweetener contains 40 mg of aspartame
- Cereal based desert = 1000 mg/kg dose of aspartame
- Water based flavoured drink = 600 mg/l dose of aspartame
- Chewing gum with no added sugar = 5500 mg/kg dose of aspartame
- Fat based dessert (no added sugar) = 1000 mg/kg dose of aspartame

All Cans/Glasses/Cups/Bottles assumed to contain water based flavoured drink

Aside from the general assumptions made above, in some instances it was necessary to make more specific assumptions on individual cases; these specific assumptions are annotated against each case report in the spreadsheet used to populate the database.

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ABBREVIATIONS

%ile – Percentile

ADI – Acceptable Daily Intake

ANS – Food Additives and nutrient sources added to food

BMI – Body Mass Index

bw – Body Weight

CDC – Centre for Disease Control and Prevention

DBPCFC - Double-Blind, Placebo-Controlled Food Challenge

EFSA - European Food Safety Authority

FDA – Food and Drug Administration (United States)

FSA – Food Standards Agency

FSANZ – Food Standards Australia New Zealand

ICD - International Classification of Diseases

JECFA – Joint Expert Committee on Food Additives

LOAEL – Lowest Observed Adverse Effect Level

NOAEL – No Observable Adverse Effect Level

SCF - Scientific Committee on Food

SPT – Skin Prick test

TOR – Terms of Reference

VKM – Vitenskapskomiteen for mattrygghet (Norwegian Scientific Committee for Food Safety)

VWA – Voedsel en Waren Autoriteit (Netherlands Food and Consumer Product Safety Authority)

WHO – World Health Organisation