

1 **ENDORSED FOR PUBLIC CONSULTATION**

2 **DRAFT SCIENTIFIC OPINION**

3 **DRAFT Scientific Opinion on the re-evaluation of aspartame (E 951) as a**
4 **food additive¹**

5 **EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)^{2,3}**

6 European Food Safety Authority (EFSA), Parma, Italy

7 **ABSTRACT**

8 The Panel on Food Additives and Nutrient Sources added to Food provides a scientific opinion re-
9 evaluating the safety of aspartame (E951). Aspartame (E 951) is an artificial sweetener authorised as a
10 food additive in the EU that was previously evaluated by JECFA, SCF and EFSA. JECFA and SCF
11 established an ADI of 40 mg/kg bw/day. The Panel based its evaluation on original reports, previous
12 evaluations, additional literature available since these evaluations and the data available following a
13 public call for data. Aspartame is rapidly and completely hydrolysed in the gastrointestinal tract to
14 methanol and the amino acids phenylalanine and aspartic acid. DKP is a degradation product of
15 aspartame. The Panel concluded that chronic toxicity and reproductive and developmental toxicity
16 were the critical endpoints in the animal database. The Panel considered that the evaluation of long-
17 term effects of aspartame should continue to be based upon the animal data. Based on a MoA analysis
18 and the weight-of-evidence, the Panel considered that the reproductive and developmental toxicity in
19 animals was due to phenylalanine released from aspartame and concluded that the basis for evaluation
20 of the reproductive and developmental endpoint should be the available data in humans. From the
21 aspartame dose-plasma phenylalanine concentration response modelling, the Panel considered that
22 aspartame intakes up to the ADI of 40 mg/kg bw/day in addition to phenylalanine from a meal would
23 not lead to peak plasma phenylalanine concentrations above the current clinical guideline for
24 prevention of adverse effects in the fetuses of PKU mothers. The Panel concluded that there were no
25 safety concerns at the current ADI of 40 mg/kg bw/day. Therefore, there was no reason to revise the
26 ADI for aspartame. Conservative estimates of exposure to aspartame and its degradation product DKP
27 made by the Panel for the general population were below their respective ADIs.

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29

30 **KEY WORDS**

31 Aspartame, E 951, methanol, artificial sweetener, EINECS number 245-261-3

32

33 **SUMMARY**

34 Following a request from the European Commission, the Panel on Food Additives and Nutrient
35 Sources added to Food (ANS) of the European Food Safety Authority (EFSA) was asked to deliver a
36 scientific opinion on the re-evaluation of aspartame (E 951) as a food additive.

37 Aspartame (E 951) is an artificial non-saccharide sweetener authorised as a food additive in the EU
38 that was previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives
39 (JECFA), the EU Scientific Committee for Food (SCF) and the European Food Safety Authority
40 (EFSA). Both JECFA and SCF established an Acceptable Daily Intake (ADI) of 40 mg/kg body
41 weight (bw)/day.

42 The Panel was not provided with a newly submitted dossier and based its evaluation on previous
43 evaluations, additional literature that has become available between then and the end of November
44 2012 and the information submitted following a public call for data. The Panel noted that although
45 many of the studies were old and were not performed according to current standards (e.g. Good
46 Laboratory Practice (GLP) and Organisation for Economic Co-operation and Development (OECD)
47 guidelines), they should be considered in the re-evaluation of the sweetener as long as the design of
48 such studies and the reporting of the data were considered appropriate. In its re-evaluation of
49 aspartame, the Panel also considered the safety of its metabolites methanol, phenylalanine and
50 aspartic acid and of its degradation products 5-benzyl-3,6-dioxo-2-piperazine acetic acid (DKP) and
51 β -aspartame, which also may be present in the sweetener as impurities.

52 Aspartame (E 951) is a dipeptide of L-phenylalanine methyl ester and L-aspartic acid bearing an
53 amino group at the α -position from the carbon of the peptide bond (α -aspartame). The major
54 degradation products of aspartame are L-phenylalanine, aspartic acid, methanol and DKP. DKP is
55 formed through the intramolecular reaction of the primary amine with the methyl ester of aspartame.
56 β -Aspartame is a non-sweet isomer of α -aspartame.

57 Specifications have been defined in the European Commission Regulation (EU) No 231/2012 and by
58 JECFA.

59 Studies in experimental animals and humans have shown that after oral ingestion, aspartame is fully
60 hydrolysed within the gastro-intestinal tract. The products resulting from these reactions are methanol
61 and the amino acids aspartic acid and phenylalanine. Hydrolysis of aspartame releases a
62 corresponding 10% by weight of methanol. Hydrolysis is very efficient and the amount of intact
63 aspartame that enters the bloodstream has been reported as undetectable in several studies conducted
64 in various species, including rats, dogs, monkeys and humans. Further studies conducted in monkeys
65 and pigs have shown that also the potential intermediate metabolite, phenylalanine methyl ester, is
66 rapidly broken down to phenylalanine and methanol in the gastro-intestinal tract. Therefore, the Panel
67 considered that phenylalanine, aspartate and methanol are absorbed and enter normal endogenous
68 metabolic pathways.

69 The acute toxicity of aspartame was tested in mice, rats, rabbits and dogs and was found to be very
70 low. Similarly, sub-acute and sub-chronic studies did not indicate any significant toxic effects in rats,
71 mice or dogs.

72 Aspartame has been tested for genotoxicity in a number of *in vitro* and *in vivo* studies. The Panel
73 concluded that in mammalian systems, apart from a valid UDS study, which was negative, no
74 conclusion could be drawn at the gene and chromosomal level, as no studies dealing with these
75 endpoints were available. However, the Panel considered that the weight-of-evidence was sufficient to
76 conclude that aspartame was not mutagenic in bacterial systems. *In vivo*, the majority of investigations
77 on systemic genotoxicity reported negative findings. Equivocal findings were only described in one

78 NTP study, positive in female but not in male p53 haploinsufficient mice but in two other transgenic
79 mouse strains the genotoxicity results were negative. The available *in vitro* data did not indicate a
80 direct genotoxic activity of aspartame that might predispose to a site of first contact effect *in vivo*.
81 Overall, the Panel concluded that the available data do not indicate a genotoxic concern for aspartame.

82 The results from three chronic toxicity and carcinogenicity studies in rats and one in mice revealed no
83 aspartame-related increase in any type of neoplasms at all doses tested. The incidences of intracranial
84 neoplasms observed in some studies were within the range of spontaneous brain tumours observed in
85 the strain of rats used. In the rat studies the highest doses tested (4000 or 8000 mg aspartame/kg
86 bw/day) produced minor renal changes considered by the Panel to be of minimal toxicological
87 significance. A dose-dependent depression of body weight gain at 2000 and 4000 mg/kg bw/day
88 correlated with decreased feed consumption was reported in one study. Overall, the Panel derived a no
89 observable adverse effect level (NOAEL) of 4000 mg/kg bw/day from the four studies.

90 Furthermore, the US NTP (US National Toxicology Program) carried out several 9-month
91 carcinogenicity studies with aspartame in genetically modified Tg.AC hemizygous, p53
92 haploinsufficient and Cdkn2a deficient mice. The Panel agreed that there was no evidence of
93 treatment-related neoplastic or non-neoplastic lesions in any of these studies.

94 Since the last evaluation of aspartame by the SCF in 2002, two new long term carcinogenicity studies
95 on aspartame in rats and one in mice were published (Soffritti *et al.*, 2006, 2007, 2010). The two rat
96 studies have already been evaluated by the former Scientific Panel on Food Additives, Flavourings,
97 Processing Aids and Materials in Contact with Food (AFC) and the ANS Panel and were considered
98 to have methodological flaws, in addition to a high background incidence of chronic inflammatory
99 changes in the lungs and other vital organs and tissues and the uncertainty about the correctness of the
100 diagnoses of some tumour types, which rendered the validity of the findings questionable. Moreover,
101 EPA has recently concluded that many of the malignant neoplasms and the lymphoid dysplasias
102 diagnosed in the studies from the Soffritti laboratory were hyperplasias related to unknown chronic
103 infection of the animals and not related to aspartame intake. Furthermore, in the mouse study, the
104 ANS Panel noted that the hepatic and pulmonary tumour incidences reported fell within the institute's
105 own historical control ranges for spontaneous tumours.

106 The available reproductive and developmental toxicity studies on aspartame comprised nine studies:
107 an embryotoxicity and teratogenicity study performed in the mouse, a two-generation reproduction
108 toxicity study in the rat, five perinatal and postnatal developmental studies in the rat, a reproductive
109 performance and developmental study in the rat and an embryotoxicity and teratogenicity study in the
110 rat. In addition, eight embryotoxicity and teratogenicity studies were performed in the rabbit, four
111 with administration of aspartame by diet and four by gavage.

112 From the rodent studies, the Panel identified a NOAEL of 5700 mg/kg bw/day, the highest dose level
113 tested, in a developmental toxicity study in the mouse. The results of the reproductive and
114 developmental toxicity studies in rats indicated NOAELs that ranged from 2000 mg aspartame/kg
115 bw/day to 4000 mg/kg bw/day. The Panel noted that where developmental changes in pup body
116 weight were observed at birth in studies at the dose of 4000 mg aspartame/kg bw/day, these might be
117 attributed to a combination of malnutrition and nutritional imbalance probably as a consequence of
118 excessive intake of phenylalanine, a breakdown product of aspartame. In support of this hypothesis,
119 the Panel noted that administration of a dose of L-phenylalanine equimolar to aspartame led to a
120 similar decrease in maternal and pup body weight as that observed in a concurrent aspartame group.

121 Several reproductive and developmental toxicity studies performed in rabbits, where aspartame was
122 administered via the diet or by gavage, were available to the Panel. Overall, the Panel considered that
123 the data from these studies were confounded both by the decrease in feed intake (when aspartame was
124 administered via the diet or by gavage), or the poor health of the animals, and, in many cases, by the

125 number of deaths of pregnant rabbits in the treated groups possibly related to misdosing during
126 gavage treatment. In one particular study with aspartame, pregnant rabbits were also dosed by gavage
127 with L-phenylalanine and L-aspartic acid groups at dose levels equimolar to the top dose of 2000 mg
128 aspartame/kg bw tested. A decrease in feed consumption was observed in the high aspartame dose and
129 the L-phenylalanine-treated animals in this study and a significant body weight loss in the high
130 aspartame dose was reported. Abortions were observed in the high dose aspartame group and to a
131 lesser extent in the L-phenylalanine group compared to no abortions in the controls. Mean fetal body
132 weight and length were significantly reduced in both the high aspartame group and the L-
133 phenylalanine group animals and a significantly higher rate of total (major and minor) malformations
134 in the 2000 mg aspartame/kg bw/day group animals as compared to concurrent control group was
135 reported. The Panel considered the possibility that, in addition to a reduced feed intake by the mothers
136 and gastrointestinal disturbances, exposure to high levels of aspartame-derived phenylalanine may be
137 in part responsible for these effects in the high dose aspartame group because similar effects, though
138 less severe, were seen in the phenylalanine group. Based on the above considerations the Panel
139 identified a NOAEL of 1000 mg aspartame/kg bw/day for maternal (weight loss) and developmental
140 toxicity (weight loss and malformations).

141 The Panel considered there was no epidemiological evidence for possible associations of aspartame
142 with various cancers in the human population. A large prospective cohort study in Denmark found a
143 small but significantly elevated risk of medically induced pre-term delivery in women with higher
144 reported consumption of artificially sweetened drinks (but not aspartame specifically). However,
145 another prospective study in Norway found that the association of pre-term delivery with artificially
146 sweetened soft drinks was much weaker and barely discernible, and applied more to spontaneous than
147 medically induced deliveries and was exceeded by an association with consumption of sugar-
148 sweetened soft drinks.

149 Methanol is a metabolite of aspartame and is subject to significant first pass metabolism. The main
150 route of metabolism of methanol proceeds by stepwise oxidation via formaldehyde to formate and
151 then to carbon dioxide. Formate can also enter the one-carbon metabolic pool. Since some authors
152 (Soffritti *et al.*, 2010; Halldorsson *et al.*, 2010) have suggested that methanol is responsible for the
153 potential carcinogenicity and toxicity of aspartame, the Panel evaluated the available toxicological
154 information on methanol.

155 The Panel considered the database on the genotoxicity of methanol and concluded that the data set
156 was limited but that the available reliable *in vitro* and *in vivo* data did not indicate a genotoxic
157 potential of methanol.

158 The oral studies on chronic toxicity and carcinogenicity of methanol are limited to a mouse study and
159 a rat study. Overall, the Panel concluded that both the mouse study (Apaja, 1980) and the rat study
160 (Soffritti *et al.*, 2002) did not contribute to the assessment of the carcinogenic potential of methanol.

161 The reproductive and developmental toxicity database of methanol is limited. From studies in mice,
162 the Panel noted that 4000 mg/kg bw/day could be a Lowest Observed Adverse Effect Level (LOAEL)
163 for methanol in mice. The Panel concluded that the data on reproductive and developmental toxicity
164 did not suggest that there was a risk from methanol derived from aspartame at the current exposure
165 estimates or at the ADI of 40 mg/kg bw/day.

166 Another aspartame metabolite, aspartic acid is itself a neurotransmitter and can be converted to the
167 more potent excitatory neurotransmitter glutamate. The Panel did not see any evidence of
168 neurotoxicity associated with aspartame. The Panel concluded that aspartic acid generated from
169 aspartame was not of safety concern at the current exposure estimates or at the ADI of 40 mg/kg
170 bw/day.

171 Concerning the third aspartame metabolite, phenylalanine, the Panel concluded that it is the main
172 metabolite of concern in terms of potential developmental effects in humans. The Panel considered
173 that it was plausible that phenylalanine could be responsible for some or all of the adverse effects
174 reported for aspartame in animal studies. The Panel considered that this was particularly true for the
175 rat and rabbit developmental toxicity studies.

176 Humans heterozygous for phenylalanine hydroxylase mutations, show a slightly reduced capacity to
177 metabolise phenylalanine compared to normal individuals. Individuals homozygous for phenylalanine
178 hydroxylase mutations (Phenylketonuria (PKU) patients) have a markedly reduced capacity for
179 phenylalanine metabolism. After birth, they show severe developmental and cognitive effects if the
180 phenylalanine intake via the diet is not strictly controlled. PKU homozygous mothers with poorly
181 controlled phenylalanine intake in their diet during pregnancy may give birth to babies with
182 congenital heart diseases, microcephalus and impaired neurological function.

183 The Panel considered that adverse effects reported for aspartame in animal studies could be attributed
184 to the metabolite phenylalanine, which was particularly the case for the rat and rabbit developmental
185 toxicity studies. The Panel noted that adverse developmental effects were seen in children born to
186 PKU patients and that these effects appeared to be related to maternal phenylalanine levels. The Panel
187 was aware that the knowledge on effects of phenylalanine in PKU mothers and their children both
188 before and after birth had developed considerably since the initial evaluation of aspartame.

189 The Panel undertook a formal Mode of Action (MoA) analysis of the putative role of phenylalanine in
190 the developmental toxicity seen in animal studies. This MoA analysis is described in Section 11.

191 The Panel considered that the proposed MoA is plausible and relevant based on the weight-of-
192 evidence of the available data summarised in the opinion. There are uncertainties from the limited
193 kinetic data in animals and in the human aspartame dose-phenylalanine concentration response data.
194 The Panel decided to base the risk characterisation on comparison of plasma phenylalanine levels
195 following aspartame administration with plasma phenylalanine levels associated with developmental
196 effects in children born from PKU mothers. The Panel decided these human data were more
197 appropriate than the results of animal studies of reproductive and developmental toxicity for the risk
198 characterization of aspartame.

199 Having established that the MoA was plausible and relevant, the Panel reviewed the information on
200 plasma levels of phenylalanine associated with adverse effects on the fetuses of PKU mothers. The
201 Panel noted that current clinical guidelines recommend that plasma levels of phenylalanine should be
202 maintained below 360 μM . In calculating a safe level of aspartame exposure (based on plasma
203 phenylalanine concentrations), the Panel assumed the worst-case scenario that intake of aspartame
204 occurs in combination with the meal which leads to circulating plasma phenylalanine concentrations
205 of 120 μM (the maximum plasma concentration based on conservative assumptions of dietary
206 exposure to phenylalanine). The concentration of plasma phenylalanine derived from aspartame was
207 therefore set to 240 μM (i.e. 360 μM minus 120 μM) by the Panel. Based on the modelling, a plasma
208 phenylalanine concentration of 240 μM would result from the administration of a bolus dose of 103
209 mg aspartame/kg bw (lower bound distributions: 88 mg aspartame/kg bw, 95th percentile, CI 59-125)
210 to a normal subject. For a PKU heterozygous individual the concentration of 240 μM would be
211 reached by the administration of a bolus dose of 59 mg aspartame/kg bw (lower bound distributions:
212 50 mg aspartame/kg bw, 95th percentile, CI 28-69). The Panel considered that given the conservative
213 assumptions and the confidence intervals provided by the modelling, for realistic dietary intake of
214 aspartame, the peak plasma phenylalanine levels would not exceed 240 μM .

215 The Panel noted that in the normal population the 95th percentile confidence interval for the lower
216 bound of the dose resulting in a peak plasma level of 240 μM following a bolus administration of
217 aspartame was greater than 40 mg/kg bw (the current ADI). In the PKU heterozygous population the

218 95th percentile confidence interval for the lower bound of the dose resulting in a peak plasma level of
219 240 µM following a bolus administration of aspartame was greater than 40 mg/kg bw (the current
220 ADI) in 82% of the simulations. The Panel considered that following bolus administration of
221 aspartame of 40 mg/kg bw (the current ADI) the PKU heterozygous population would not exceed the
222 current clinical guideline of 360 µM.

223 The Panel also noted that in order to exceed the phenylalanine plasma concentration of 240 µM
224 following repeated administration of aspartame in normal individuals, a bolus administration at 40
225 mg/kg bw (equivalent to the current ADI) would need to be given every hour.

226 The Panel considered the following:

- 227 • the conservative assumptions used in the modelling, which would all overestimate peak
228 plasma concentrations
- 229 • the available information on adverse effects on development in humans with PKU
- 230 • comparison with a concentration of 240 µM to allow for simultaneous ingestion of
231 phenylalanine from other components of the diet in order to not exceed the current clinical
232 guideline of 360 µM
- 233 • results of the modelling
- 234 • data from repeated oral administration of aspartame in humans
- 235 • bolus intakes based on consumption of one litre of soft drink containing aspartame at the
236 maximum permitted level (MPL) of 600 mg/L by a child of 20-30 kg are unlikely to exceed
237 30 to 20 mg/kg bw, respectively

238 Based on these considerations and evaluations, the Panel concluded that under realistic conditions,
239 phenylalanine plasma levels would not exceed 240 µM in normal or PKU heterozygous individuals.
240 The Panel noted that this was considerably far below the concentrations at which adverse effects in
241 the fetus are reported and is also below the current clinical guideline (360 µM) for prevention of
242 effects in the fetuses of pregnant PKU patients. The Panel noted that in young children who did not
243 suffer from PKU, plasma levels of phenylalanine resulting from aspartame ingestion at or below the
244 ADI (as either a bolus or other aspartame consumption patterns) were likely to remain below 240 µM.
245 For pregnant women, the Panel noted that there was no risk to the fetus from phenylalanine derived
246 from aspartame at the current ADI (40 mg/kg bw/day) in normal or PKU heterozygous individuals.

247 The Panel noted that it was currently not possible to include chronic endpoints in the postulated MoA.
248 The Panel noted that the ADI previously derived by JECFA and SCF of 40 mg/kg bw/day appeared to
249 be based on the long-term animal studies using the default uncertainty factor of 100. The Panel
250 considered that this remained appropriate for the evaluation of long-term effects of aspartame.

251 The current evaluation was based on analysis of human reproductive and developmental effects of
252 phenylalanine in PKU patients, who are more susceptible than the general and PKU heterozygous
253 population. Therefore, no additional allowance for toxicodynamic variability was required. The
254 modelling of the aspartame dose-phenylalanine concentration response was based on data from PKU
255 heterozygous individuals who at any dose would have a higher plasma phenylalanine concentration
256 than the normal population; therefore, no additional allowance for toxicokinetic variability was
257 required. The Panel concluded that exposures at or below the current ADI were not of safety concern
258 for reproductive and developmental toxicity in humans excluding PKU homozygous individuals.

259 The Panel concluded from the present assessment of aspartame that there were no safety concerns at
260 the current ADI of 40 mg/kg bw/day. Therefore, there was no reason to revise the ADI for aspartame.

261 The Panel emphasised that its evaluation of phenylalanine plasma levels from a dose of aspartame at
262 the ensuing ADI is not applicable to PKU patients. These individuals require total control of dietary
263 phenylalanine intake to manage the risk from elevated phenylalanine plasma levels. The Panel noted
264 that risk managers have recognised this by requiring appropriate labelling to indicate that aspartame is
265 a source of phenylalanine.

266 Conservative estimates of exposure to aspartame made by the Panel for the general population were
267 up to 36 mg/kg bw/day at the 95th percentile. These were below the ADI. The current ADI for DKP is
268 7.5 mg/kg bw/day. Estimates of DKP exposure at the ADI for aspartame are below this ADI based on
269 the current specification for DKP in aspartame (1.5%). The Panel noted that high-level exposure
270 estimates for the general population are up to 5.5 mg/kg bw/day at the 95th percentile, which is below
271 the ADI. Finally, conservative estimates of exposure to methanol showed that aspartame-derived
272 methanol contributed to less than 10% of the total mean anticipated exposure to methanol from all
273 sources.

274

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465 **BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION**

466
467 Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives
468 requires that food additives are subject to a safety evaluation by the European Food Safety Authority
469 (EFSA) before they are permitted for use in the European Union. In addition, it is foreseen that food
470 additives must be kept under continuous observation and must be re-evaluated by EFSA.

471 For this purpose, a programme for the re-evaluation of food additives that were already permitted in
472 the European Union before 20 January 2009 has been set up under the Regulation (EU) No 257/2010⁴.
473 This Regulation also foresees that food additives are re-evaluated whenever necessary in light of
474 changing conditions of use and new scientific information. For efficiency and practical purposes, the
475 re-evaluation should, as far as possible, be conducted by group of food additives according to the main
476 functional class to which they belong.

477 The order of priorities for the re-evaluation of the currently approved food additives should be set on
478 the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific
479 Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of
480 a food additive in food and the human exposure to the food additive taking also into account the
481 outcome of the Report from the Commission on Dietary Food Additive Intake in the EU⁵ of 2001. The
482 report 'Food additives in Europe 2000'⁶ submitted by the Nordic Council of Ministers to the
483 Commission, provides additional information for the prioritisation of additives for re-evaluation. As
484 colours were among the first additives to be evaluated, these food additives should be re-evaluated
485 with a highest priority.

486 In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised
487 food additives. However, as a result of adoption of Regulation (EU) 257/2010 the 2003 Terms of
488 References are replaced by those below.

489 **TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION**

490 The Commission asks the European Food Safety Authority to re-evaluate the safety of food additives
491 already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking
492 especially into account the priorities, procedures and deadlines that are enshrined in the Regulation
493 (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food
494 additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the
495 Council on food additives.

496

⁴ OJ L 80, 26.03.2010, p19

⁵ COM(2001) 542 final.

⁶ Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers, TemaNord 2002:560.

497 **ASSESSMENT**

498 **1. Introduction**

499 The present opinion deals with the re-evaluation of the safety of aspartame (E 951) when used as an
 500 artificial sweetener added to foods, and including its use as a tabletop sweetener.

501 Aspartame is an artificial non-saccharide sweetener authorised as a food additive in the EU that was
 502 previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the
 503 latest in 1981 (JECFA, 1975; 1980; 1981), the EU Scientific Committee for Food (SCF), the latest in
 504 2002 (SCF 1985; 1989; 1997; 2002), the European Food Safety Authority (EFSA), the latest in 2011
 505 (EFSA, 2006; 2009a; 2009b; 2011a; 2011b).

506 The Panel was not provided with a newly submitted dossier and based its evaluation on previous
 507 evaluations, additional literature that became available between then and the end of November 2012
 508 and the data submitted following a public call for data⁷. The selection criteria for scientific data
 509 consideration for the re-evaluation of aspartame described in this opinion applied to both the existing
 510 published and unpublished scientific literature. These criteria were agreed at the 28th Scientific Panel
 511 on Food Additives and Nutrient Sources added to Food (ANS) Plenary meeting on 25-27 October
 512 2011 and were published with the minutes of that meeting. These criteria are reproduced in Annex A.

513 In its re-evaluation of aspartame, the ANS also considered the safety of its metabolites methanol,
 514 phenylalanine and aspartic acid and its degradation products 5-benzyl-3,6-dioxo-2-piperazine acetic
 515 acid (DKP) and β -aspartame, which also may be present in the sweetener as an impurity.

516 The Panel noted that in the early studies, the stereochemical forms of the amino acids and other
 517 metabolites formed from aspartame were not specified.

518 **2. Technical data**

519 **2.1. Identity of the substances**

520 Aspartame is a dipeptide of L-phenylalanine methyl ester and L-aspartic acid bearing an amino group
 521 at the α -position from the carbon of the peptide bond (Figure 1).

522 Aspartame has a molecular formula of C₁₄H₁₈N₂O₅. It has a molecular weight of 294.31 g/mol, the
 523 CAS Registry Number is 22839-47-0 and the EINECS number is 245-261-3. The chemical name for
 524 aspartame is (S)-3-amino-N-[(S)-1-methoxycarbonyl-2-phenylethyl] succinamic acid. The structural
 525 formula of aspartame is presented in Figure 1.

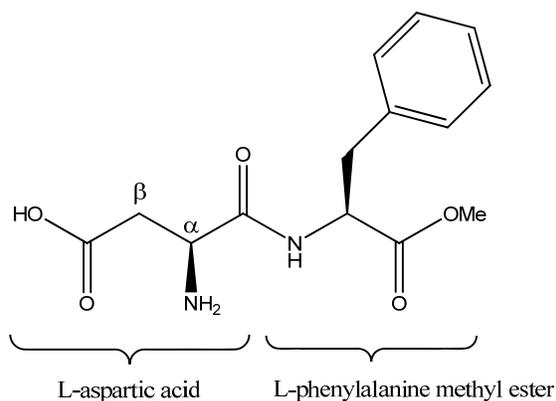
526 Aspartame is a white odourless, crystalline powder having a sweet taste.

527 It has been reported that at pH 7 and room temperature, aspartame has a solubility in water of
 528 approximately 10 g/l (Homler *et al.*, 1991). According to data from a producer, aspartame has a
 529 solubility in ethanol of 0.37% and is insoluble in oil.⁸ The theoretical log P_{o/w} value calculated with
 530 Advanced Chemistry Development Labs software (ACD Labs) is 0.542 \pm 0.626 (at 25°C). Aspartame
 531 has two melting points, melting initially at 190°C, solidifying and re-melting at 246-247°C. A possible
 532 explanation for this behaviour is that the intramolecular reaction of the primary amine with the methyl
 533 ester takes place at the initial melting point to generate 5-benzyl-3,6-dioxo-2-piperazine acetic acid
 534 (DKP) and methanol (Pranker, 2002).

⁷ 'Call for scientific data on Aspartame (E951)' (published: 1 June 2011). Available from:
<http://www.efsa.europa.eu/en/dataclosed/call/110601.htm>

⁸ <http://www.nutrasweet.com/articles/sendfile.asp?Id=130&filename=AG%2DTB%2D03%2D001%2Epdf>

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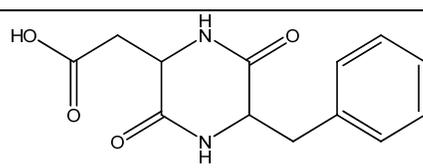
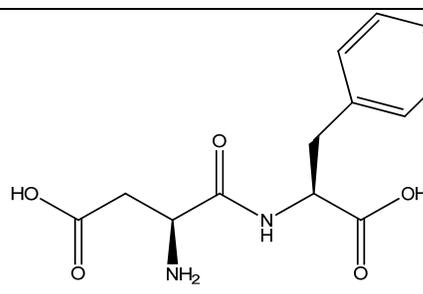
Figure 1: Structural formula of aspartame E 951 (α -aspartame).

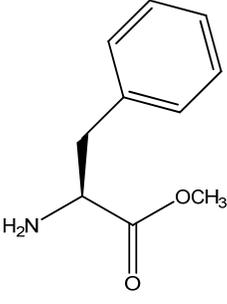
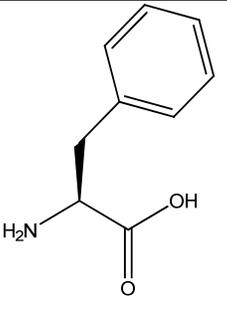
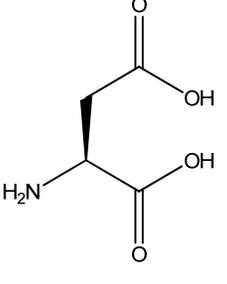
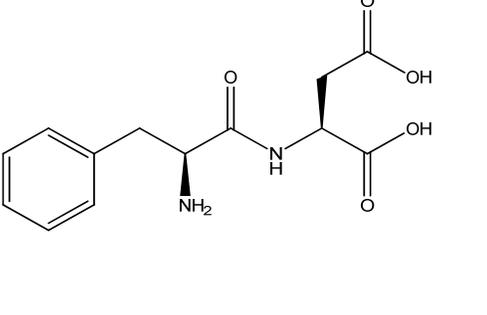
538 Some of the synonyms of aspartame include α -aspartame, α -APM, N-L-alpha-aspartyl-L-
539 phenylalanine-1-methyl ester, aspartyl phenylalanine methyl ester, L-aspartyl-L-phenylalanyl methyl
540 ester, L-aspartyl-L-3-phenylalanine methyl ester, L-alpha-aspartyl-L-phenylalanine methyl ester,
541 alpha-L-aspartyl-L-phenylalanine methyl ester, methyl aspartylphenylalanate, L-aspartame, alpha-
542 aspartame.

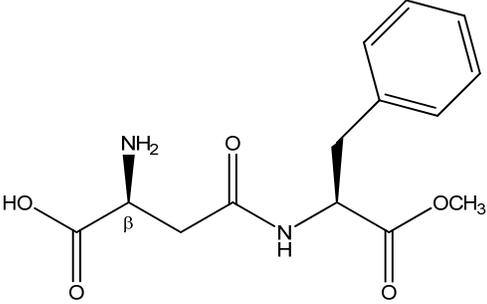
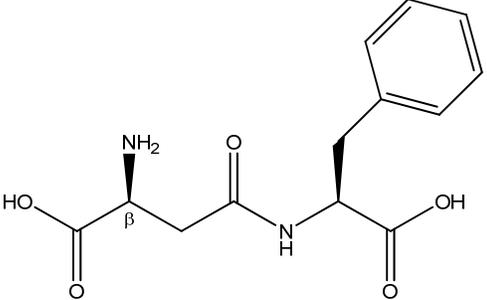
543 Identity of degradation products of aspartame

544 The major degradation products of aspartame are listed in Table 1.

545 **Table 1:** Major degradation products of aspartame

Name	CAS Registry Number	Structural formula
5-benzyl-3,6-dioxo-2-piperazine acetic acid (DKP)	55102-13-1 (stereochemistry not specified) 5262-10-2 (configuration 2S,5S-)	
Methanol	67-56-1	CH ₃ OH
L-Aspartyl-L-phenylalanine (α -AP)	13433-09-5	

<i>Name</i>	<i>CAS Registry Number</i>	<i>Structural formula</i>
L-Phenylalanine methyl ester	2577-90-4	
L-Phenylalanine (Phe)	63-91-2	
L-Aspartic acid (Asp)	56-84-8	
L-Phenylalanine-L-aspartic acid		

Name	CAS Registry Number	Structural formula
β-Aspartame (β-APM)	2577-90-4	
N-L-β-Aspartyl-L-phenylalanine (β-AP)		

546

547 **5-benzyl-3,6-dioxo-2-piperazine acetic acid (DKP)**

548 The intramolecular reaction of the primary amine with the methyl ester forms DKP and methanol. The
549 molecular formula of DKP is C₁₃H₁₄N₂O₄. Its molecular weight is 262.26 g/mol.

550 The log P_{o/w} value is -0.269 ± 0.515 at 25°C, the pK_{a1} is 4.02 ± 0.10 and pK_{a2} is -1.55 ± 0.60 both at
551 25°C, values calculated with Advanced Chemistry Development (ACD/Labs).

552 The synonyms for DKP include 3-benzyl-6-(carboxymethyl)-2,5-diketopiperazine, 3-benzyl-6-
553 carboxymethyl-2,5-dioxopiperazine, 3-carboxymethyl-6-benzyl-2,5-diketopiperazine, cyclo(aspartyl-
554 phenylalanyl) and 5-benzyl-3,6-dioxo-2-piperazineacetic acid (5BZ).

555 DKP can degrade by hydrolysis of one of the amides in the diketopiperazine moiety to phenylalanine
556 aspartic acid or α-aspartylphenylalanine (see Table 1). The stereochemistry of the compounds is not
557 given due to possible racemisation of aspartic acid and phenylalanine moieties in DKP (Boehm and
558 Bada, 1984). Both intermediates hydrolyse to yield the amino acids aspartic acid and phenylalanine⁹
559 (Prodolliet and Bruelhart, 1993; Langguth *et al.*, 1991).

560 **β-Aspartame**

561 β-Aspartame is non-sweet. Its chemical name is N-L-β-aspartyl-L-phenylalanine 1-methyl ester.

⁹ The term phenylalanine and phenylalanine methyl ester are used when the stereochemistry of the compounds is not specified in the original reference.

562 **2.2. Specifications**

563 Specifications for aspartame according to Commission Regulation (EU) No 231/2012¹⁰ and to JECFA
564 (2006) are listed in Table 2. The purity is specified as not less than 98% and not more than 102%.

565 **Table 2:** Specifications for aspartame according to Commission Regulation (EU) No 231/2012 and
566 JECFA (2006)

	Commission Regulation (EU) 231/2012	JECFA (2006)
Assay	Not less than 98% and not more than 102% of C ₁₄ H ₁₈ N ₂ O ₅ on the anhydrous basis	Not less than 98% and not more than 102% on the dried basis
Description	White odourless, crystalline powder having a sweet taste. Approximately 200 times as sweet as sucrose	White, odourless, crystalline powder, having a strong sweet taste
Identification		
Solubility	Slightly soluble in water and in ethanol	Slightly soluble in water and in ethanol
Test for amine group	-	Dissolve 2 g of ninhydrin in 75 ml of dimethyl sulphoxide, add 62 mg of hydrindantin, dilute to 100 ml with 4 M lithium acetate buffer solution (pH 9) and filter. Transfer about 10 mg of the sample to a test tube, add 2 ml of the reagent solution, and heat. A dark purple colour is formed.
Test for ester	-	Dissolve about 20 mg in 1 ml of methanol, add 0.5 ml of methanol saturated with hydroxylamine hydrochloride, mix, and then add 0.3 ml of 5 N potassium hydroxide in methanol. Heat the mixture to boiling, then cool, adjust the pH to between 1 and 1.5 with hydrochloric acid TS, and add 0.1 ml of ferric chloride TS. A burgundy colour is produced.
pH (1 in 125 solution)	4.5 and 6.0	4.5-6.0
Specific rotation (Determine in a 4 in 100/15 N formic acid solution within 30 minutes after preparation of the sample solution)	$[\alpha]_D^{20}$: +14.5 to + 16.5 °	$[\alpha]_D^{20}$: +14.5 to + 16.5 °
Purity		
Loss on drying (105°C, 4 hours)	Not more than 4.5%	Not more than 4.5%
Sulphated ash	Not more than 0.2% (expressed on dry weight basis)	Not more than 0.2% Test 1g of the sample (Method I)

¹⁰ Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council OJ L 83, 22.3.2012 p 1-295.

Transmittance/ Spectrophotometry	The transmittance of a 1% solution in 2 N hydrochloric acid, determined in a 1-cm cell at 430 nm with a suitable spectrophotometer, using 2 N hydrochloric acid as reference, is not less than 0.95, equivalent to an absorbance of not more than approximately 0.022	The transmittance of a 1 in 100, 2 N hydrochloric acid solution, determined in a 1-cm cell at 430 nm with a suitable spectrophotometer, using 2 N hydrochloric acid as a reference, is not less than 0.95, equivalent to an absorbance of not more than approximately 0.022.
Arsenic	Not more than 3 mg/kg	
Lead	Not more than 1 mg/kg	Not more than 1 mg/kg
5-Benzyl-3,6-dioxo-2-piperazineacetic acid (DKP)	Not more than 1.5%	Not more than 1.5%
Other optical isomers	-	Passes test

567

568 In the JECFA specifications, aspartame is identified by an amine test and an ester test, whilst the EC
569 specifications do not include these tests. JECFA specifications analyse aspartame for the presence of
570 other optical isomers whilst the EC specifications do not include this test.

571 The Panel noted that the EC specifications for aspartame do not include a maximum limit for β -
572 aspartame, which may be formed as a by-product of the chemical synthesis of aspartame or from
573 aspartame degradation.

574 The Panel noted that the EC specifications for aspartame may need to be amended to include a
575 restriction for protein content due to the potential use of enzymes in the production of aspartame.

576 The Panel noted that both EC and JECFA specifications for aspartame are dated in terms of the tests
577 proposed for identification. These specifications should be updated to include characterisation of
578 aspartame by chromatographic and mass spectrometric methodologies.

579 2.3. Manufacturing process

580 There are several methods to produce aspartame on a commercial scale, either through chemical or
581 enzymatic synthesis (Pranker, 2002; Yagasaki and Hashimoto, 2008).

582 According to the information provided by a producer, the chemical synthesis of aspartame involves
583 the reaction of L-aspartic acid and L-phenylalanine methyl ester (Burdock Group, 2006). L-
584 phenylalanine methyl ester reacts with N-protected L-aspartic anhydride to form N-protected
585 aspartame. After re-crystallisation, the protecting group is removed to yield aspartame. Both α -
586 aspartame (the sweet isomer) and β -aspartame (the non-sweet isomer) are produced, requiring
587 separation step to obtain only α -aspartame. A similar description of the manufacturing process was
588 provided by another manufacturer that uses L-phenylalanine and N-protected L-aspartic anhydride
589 (Nutrasweet, 2012). Two major producers have indicated that the chemical synthesis is the process
590 used for their aspartame production (Ajinomoto, 2012a; Nutrasweet 2012).

591 One of the difficulties with the chemical synthesis of aspartame is avoiding the formation of the non-
592 sweet isomer (β -aspartame) which is one of the by-products.

593 Enzymatic methods allow the use of racemic starting materials, and the process has the advantage of
594 producing only α -aspartame (the sweet isomer). The enzyme typically used is thermolysin, derived
595 from *Bacillus thermoproteolyticus*. Enzymatic synthesis using protected and unprotected aspartic acid
596 and phenylalanine methyl ester are described in the literature (Yagasaki and Hashimoto, 2008). The

597 Panel noted that *Bacillus thermoproteolyticus* is not included in the list of qualified presumption of
 598 safety (QPS) biological agents intentionally added to food and feed (EFSA, 2011c).

599 **2.4. Methods of analysis in food**

600 There are a very large number of published methods for the determination of aspartame alone or in
 601 combination with other sweeteners (Zygler *et al.*, 2009). Most methods are based on extraction, with
 602 or without subsequent clean-up and high performance liquid chromatographic (HPLC) analysis using
 603 various detectors (Lino *et al.*, 2008; Ma *et al.*, 2012; Cheng and Wu, 2011; Kobayashi *et al.*, 1999; Ji
 604 *et al.*, 2009; Kang *et al.*, 2012; Serdar and Knezevic, 2011; Sik 2012) or ion chromatography (Chen
 605 and Wang, 2001). There are three EU standardised inter-laboratory validated methods for
 606 determination of aspartame in foods. Methods EN 12856:1999¹¹ and CEN/TS 15606:2009¹² employ
 607 HPLC with UV detection and EN 15911:2010¹³ uses HPLC with evaporative light scattering detector
 608 (ELSD). A validated HPLC-ELSD method has been reported for aspartame in carbonated and non-
 609 carbonated beverages, canned and bottled fruits and yoghurt (Wasik *et al.*, 2007). For samples fortified
 610 with aspartame, at the maximum permitted limits for aspartame according to the EU regulation, the
 611 method gave recoveries ranging from 90 to 100% and RSD_R values <7%. HorRat values of <1.7
 612 indicated satisfactory performance of the method for determination of aspartame in all matrices tested
 613 (Buchgraber and Wasik, 2007). Others have employed various forms of combined liquid
 614 chromatography-mass spectrometry (LC-MS) for determining aspartame in Chinese liquors (Xia *et al.*,
 615 2011), in beverages, dairy and fish products (Zygler *et al.*, 2011), juices and syrups (Ferrer and
 616 Thurman, 2010), wine (Cheng and Wu, 2011) and other foods (Yang *et al.*, 2008, Koyama *et al.*, 2005,
 617 Liu *et al.*, 2010). LC-MS methods are rapid, not requiring extensive clean-up and offer low limits of
 618 detection with high specificity, but have not to date been validated by any inter-laboratory studies.
 619 Other successful approaches for direct analysis of aspartame in commercial sweeteners have employed
 620 Fourier transform infrared spectroscopy (FT-IR) (Mazurek and Szostak, 2011), in soft drinks and
 621 tabletop sweeteners capillary electrophoresis with capacitively coupled contactless conductivity
 622 detection (CE-C⁴D) (Bergamo *et al.*, 2011) and in carbonated beverages proton nuclear magnetic
 623 resonance (¹H-NMR) (Maes *et al.*, 2012).

624 **2.5. Stability, reaction and fate in food**

625 The stability of aspartame is affected by moisture, pH, temperature and storage time.

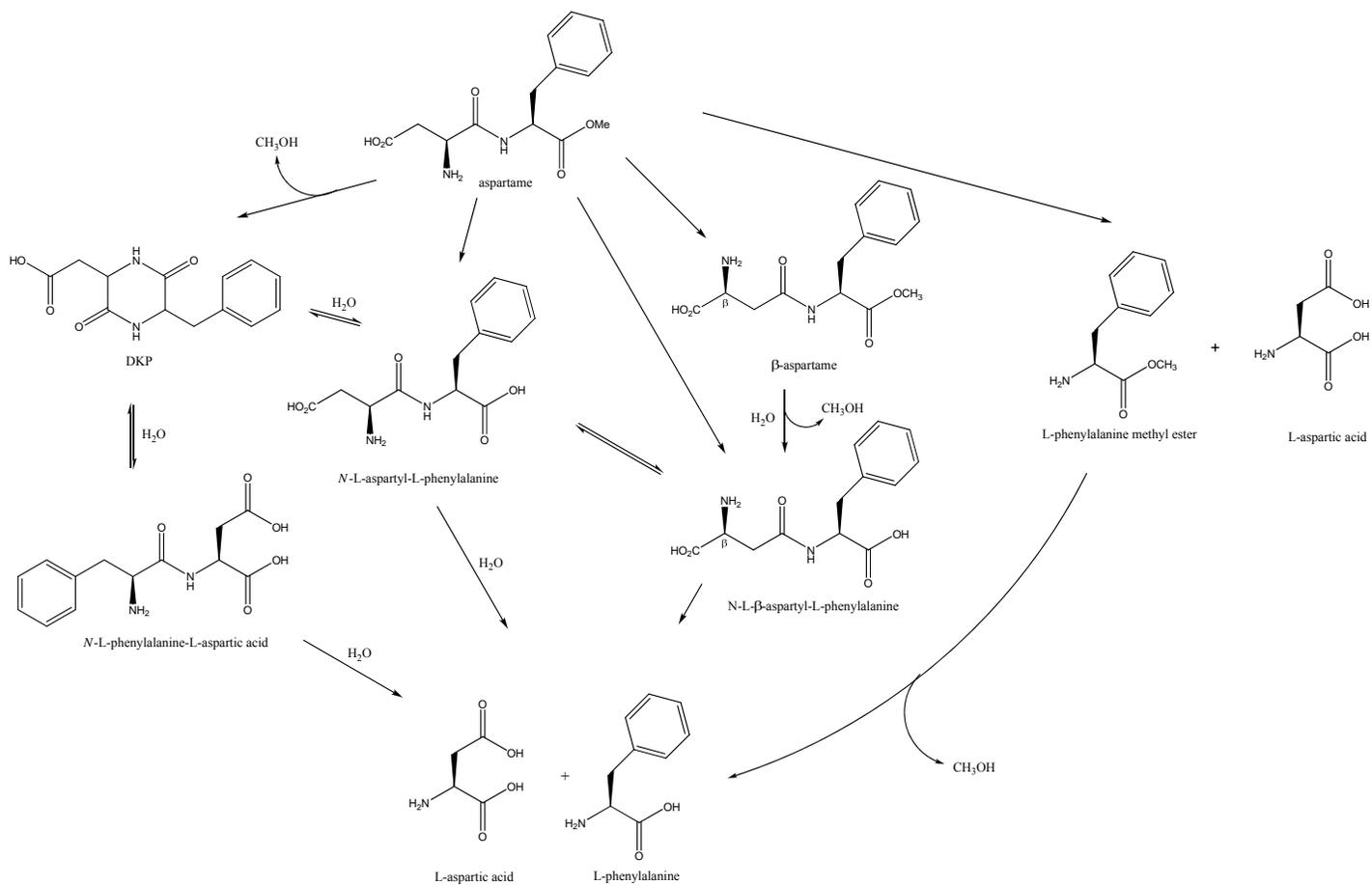
626 Figure 2 shows the degradation pathways (Bell and Labuza, 1991; Prodolet and Bruelhart, 1993). At
 627 neutral and alkaline pH, aspartame releases methanol and forms α -aspartylphenylalanine or undergoes
 628 cyclisation to form DKP. DKP and α -aspartylphenylalanine can interconvert, but do not revert to
 629 aspartame to any significant rate. Conversion of aspartame into DKP or α -aspartylphenylalanine
 630 results in the loss of the sweet taste. At acidic pHs below 4.5, in addition to the two main reaction
 631 pathways occurring at neutral and alkaline pHs, aspartame can undergo structural rearrangement to
 632 produce β -aspartame. Also the peptide bond of aspartame can be cleaved, producing L-phenylalanine
 633 methyl ester and L-aspartic acid. β -aspartylphenylalanine can be produced from the structural
 634 rearrangement of α -aspartylphenylalanine. Phenylalanine is produced by hydrolysis of phenylalanine
 635 methyl ester. Finally, α -aspartylphenylalanine can hydrolyze to the individual amino acids.

¹¹ <http://webstore.ansi.org/RecordDetail.aspx?sku=BS+EN+12856%3A1999>

¹² <https://www.astandis.at/shopV5/Preview.action;jsessionid=B20DC77D150ABD17C37DD9EEF850B4EB?preview=&dokkey=354291&selectedLocale=en>

¹³ <http://webstore.ansi.org/RecordDetail.aspx?sku=BS+EN+15911:2010>

636



637

638

Figure 2: Degradation pathways for aspartame

639 *Stability of aspartame in solid state*

640 According to industry at moisture contents of less than 8%, aspartame in its pure form is stable with a
641 minimum shelf life of 5 years (storage temperature not indicated) (Ajinomoto, 2012). Solid-state
642 stability studies showed that at temperatures higher than 80°C, aspartame cyclises with the release of
643 methanol to form DKP (Leung and Grant 1997; Lin and Cheng 2000; Conceicao *et al.*, 2005). Homler
644 reported that under dry conditions, conversion of aspartame into DKP is slow (5% per 100 hours at
645 105°C). At higher temperatures, the rate of conversion to DKP increases (around 50% per 80 hours at
646 120°C and 100% per 30 hours at 150°C) (Homler, 1984). Graves and Luo (1987) reported that when
647 heated in an acidified and dried state at 110°C for 24 hours in vacuo, in addition to the already
648 mentioned degradation products, aspartame could dehydrate to form an anhydro derivative and also
649 oligopeptides could be generated containing ratios of L-aspartic acid to L-phenylalanine that were
650 higher than the starting material.

651 Decomposition of aspartame in lyophilized instant coffee with a residual moisture content of 3-4%
652 was less than 10% after 260 days of storage (Jost *et al.*, 1982).

653 The degradation kinetics of aspartame as a function of the water activity (relative vapour pressure, a_w)
654 in low and intermediate moisture food systems showed that an increase in a_w for each 0.1 units in the
655 0.3-0.7 range, resulted in about 30-80% increase in the degradation rate (Bell and Labuza, 1991).

656 *Stability of aspartame in solution*

657 Aspartame has limited stability in solutions and undergoes pH-, temperature- and time-dependent
658 degradation (Pariza *et al.*, 1998). Extensive studies on the kinetics of aspartame degradation in
659 solution have been performed and the degradation has been reported to follow first order kinetics
660 (Langguth *et al.*, 1991).

661 Aspartame is most stable between pH 4-5 (Homler, 1984; Neiryneck and Nollet, 1988; Bell and
662 Labuza, 1991; Bell and Labuza, 1994; Sabah and Scriba, 1998; Rowe *et al.*, 2003). Rowe *et al.* (2003)
663 showed that within this range of pHs, aspartame dissolved in aqueous buffers at 25°C has a half-life >
664 250 days. When the pH was decreased to 3, the half-life was shortened to 100-125 days and further
665 decreased to below 25 days at pH 1. When the pH was increased from 5 to 6, the half-life was reduced
666 to 50-100 days and further decreased to below 25 days at pH 7. Pattanaagson *et al.* (2001) investigated
667 the degradation products of aspartame at different pH ranges. The major degradation product found a
668 pH range 2 to 6 was L-phenylalanine methyl ester, in the range of pH 7-10, the major degradation
669 product was DKP and at pH 12, it was L-aspartyl-phenylalanine.

670 In addition to the pathways discussed above, due to its free amino group, aspartame can react with
671 reducing sugars in the presence of water via the Maillard reaction. This was determined through
672 measurement of brown colour formation that resulted from mixing aspartame and glucose in solution
673 at 70, 80, 90 and 100°C at a_w of 0.8. Browning of aspartame followed zero order kinetics with the time
674 of 0.1 absorbance units at 420 nm for 11.4, 5.3, 2.15 and 1.0 hour at these respective temperatures
675 (Stamp and Labuza 1983; Huang *et al.*, 1987). The formation of a Schiff base, a step in the Maillard
676 degradation pathway, was also noted between aspartame and vanillin in methanol/water solutions (Cha
677 and Ho, 1988).

678 Aspartame is stable with respect to racemisation at mildly acidic pH values at room temperature, but at
679 100°C, racemisation of the DKP generated from aspartame takes place (Boehm and Bada 1984; Gaines
680 and Bada 1988; Gund and Veber, 1979). The authors' explanation was that DKP formation is
681 accelerated by electrostatic repulsion between the two carboxy groups which force the dipeptide into
682 the *cis* conformation. At neutral pH the relative racemisation rates of aspartame dipeptides are much
683 slower than of DKP, and amino acids at the *N*-terminal position racemise more rapidly than *C*-terminal
684 amino acids (Gaines and Bada 1988).

685 The analytical results of the electro-thermal and microwave induced hydrolysis of aspartame in cola
686 samples imply that the hydrolysis of aspartame increases with an increase in temperature, microwave
687 power and hydrolysis time. D-aspartic acid and D-phenylalanine can be observed with the electro-
688 thermal racemisation at the hydrolysis temperature of 120°C for 1 day and only D-aspartic acid can be
689 observed at the hydrolysis temperature of 90°C for 2 and 3 days. For microwave-induced hydrolysis,
690 only L-aspartic acid was detected at a power of 56 W for 1 minute and 320 W for 3 minutes (Cheng
691 and Wu, 2011).

692 The study by Lawrence and Yuan (1996) showed that aspartame, in aqueous acidic solutions in the
693 presence of ascorbic acid and a transition metal catalyst, such as copper (II) or iron (III), under aerobic
694 conditions can produce benzaldehyde via a free radical attack on aspartame. Benzaldehyde production
695 was dependent on ascorbic acid concentration, but the yield of benzaldehyde decreased as the
696 concentration of ascorbic acid exceeded that of aspartame.

697 In lime-lemon carbonated beverages with pH 3.14 - 3.21 stored for 60 days at temperatures of 4°C and
698 37°C, the loss of aspartame was 4.5% and 29.5% respectively (Malik *et al.*, 2002). In lime-lemon and
699 diet cola drinks stored at 22°C, after 6 months, 28 and 37% of the label claim of aspartame was
700 detected, respectively, and after 36 months, the detected level was reduced to 6 and 4% (Tsang *et al.*,
701 1985).

702 According to Jost *et al.* (1982), the half-life of aspartame in cola is approximately 150 days at 23°C
703 and pasteurization of orange juice results in immediate loss of approximately half of the initial
704 aspartame content.

705 Stability tests in sterilized flavoured dairy beverages with pH 6.7 showed that aspartame had a half-life
706 of 1-4 days at 30°C and 24-58 days at 4°C. Decreasing the pH from 6.7 to 6.4 doubles the half-life of
707 aspartame (Bell and Labuza, 1994).

708 Noda *et al.* (1991) demonstrated the instability of aspartame in fresh juice solutions of particular kinds
709 of fruit such as kiwi, pineapple, papaya and melon, probably by the action of proteolytic enzymes.
710 Yogurt cultures (strains of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*) degrade
711 aspartame during fermentation; however, losses of aspartame appeared related to the rate of the
712 metabolism of the cultured microorganisms. Aspartame degradation was negligible once the pH of
713 yogurt dropped below 5.0 and the culture reached a stationary phase (Keller *et al.*, 1991; Prodolliet
714 and Bruehlhart 1993).

715 *Review of DKP concentration in food from aspartame degradation*

716 The content of aspartame and its degradation products, DKP, aspartyl-phenylalanine and
717 phenylalanine, were analysed in 24 different commercial foods by Prodolliet and Bruehlhart (1993).
718 The decomposition of aspartame was particularly in high fruit cream (40%), milk chocolate (26%),
719 malted beverage (22%) and fruit yogurt (15%). It has been determined that up to 21% and 12% of the
720 aspartame in cola drink and fruit cream, respectively, is in the form of DKP.

721 The degradation of aspartame to DKP in soft drinks depends on the storage temperature. DKP
722 concentration in soft drinks after two months storage was about four times higher at a temperature
723 range of 25-27°C compared to 4-5°C (Saito *et al.*, 1989).

724 Grosse *et al.* (1991) determined the level of degradation of aspartame and the extent of the formation
725 of DKP in heat-treated dessert products. In acidified (pH 3) gelatin-based desserts, no formation of
726 DKP was observed, whereas in dessert products containing milk or cacao having a pH of 6, the
727 degradation of aspartame rose to 20-35%. According to the authors in heat-treated cacao containing
728 desserts with starch and milk as basic ingredients, at pH 6 a degradation of about 34% of the
729 aspartame to DKP can occur, whereas in heat-treated vanilla containing desserts at pH 6, about 21-
730 25% is degraded to DKP.

731 Cyprus submitted analytical data on levels of DKP in soft drinks sold in the Cyprus market. Levels
732 found range between 3.4 and 19.4 mg/L. No information on the packaging or storage conditions
733 (duration, temperature) was given (Cyprus, 2012).

734 The Panel was provided with data on the determination of aspartame and its main degradation
735 products in carbonated beverages (UN11, 1986), in baked goods (UN12, 1990), in beer (UN13), instant
736 and quick cooked oatmeal (UN14, 1987), in soft candy (UN15, 1987). Table 3 shows the concentration
737 of DKP in food from aspartame degradation observed in the above-mentioned studies as well as in the
738 available literature.

739 **Table 3:** Concentration of 5-benzyl-3,6-dioxo-2-piperazine acetic acid (DKP) in food from
740 aspartame (APM) degradation

Food	Conditions	% degraded APM	% degradation of APM to DKP	Reference
Carbonated Beverages APM initial: 0.5 g/l	pH 2.5 Time: 52 weeks T 20 ⁰ C	75	24	UN11, 1986
	pH 2.5 Time: 39 weeks T 30 ⁰ C	95	31	
	pH 4.4 Time: 38 weeks T 20 ⁰ C	21	8.3	
	pH 4.4 Time: 52 weeks T 30 ⁰ C	62	26.6	
Cheese cake (APM encapsulated) APM: 1.5 g/kg	Time: 50 min T 121 ⁰ C	16	6.4	UN12, 1990
Chocolate cake (APM encapsulated) APM: 4.5 g/kg	Time: 35 min T 177 ⁰ C	24	15	
Beer APM: 20 mg/kg	pH 4.3 Time: 90 days T 22 ⁰ C Ethanol 2.7%	8-10	5	UN13
Instant cereals APM: 1400-2000 mg/kg	Boiling water added, time in cooling water: 17 min	6	3	UN14, 1987
Instant cereals APM: 1400-2000 mg/kg	Cooked in microwave, time in cooling water: 16 min	2.5	2.3	
Gelled candy APM: 0.84 g/kg	21.1 ⁰ C Time 22 weeks	21	17	UN15, 1987
	26.6 ⁰ C Time 17 weeks	41	25	
Lime-lemon	Time 6 months T : 22 ⁰ C	43-63	17-22	Tsang <i>et al.</i> , 1985
	Time 36 months T : 22 ⁰ C	86-94	32-34	

Food	Conditions	% APM degraded	% degradation of APM to DKP	Reference
Diet Cola	Time 6 months T : 22°C	62-72	24-28	
	Time 36 months T : 22°C	96	32-35	
Diet cola APM: 20-100 µg/ml	All samples analysed were all commercial products. The samples were dissolved to yield an aspartame concentration of 20-100 µg/ml taking into account the declared values.	40	21	Prodoliet and Bruehlhart, 1993
Malted beverage APM: 20-100 µg/ml		22	3	
Fruit (prune) yogurt APM: 20-100 µg/ml		15	3	
Vanilla cream APM: 20-100 µg/ml		8	7	
Fruit cream APM: 20-100 µg/ml		40	12	
Milk chocolate APM: 20-100 µg/ml		26	7	

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The Panel noted that in the carbonated beverage study (UN11, 1986) it was observed that aspartame was also degraded to β-aspartame (β-APM) and β-aspartyl-L-phenylalanine (β-AP). At the beginning (4-12 weeks), aspartame was degraded into β-aspartame ([β-APM] > [β-AP]) and with time (>12 weeks), β-aspartame was hydrolysed to β-AP ([β-AP] >> ([β-APM])). Table 4 shows the concentration of β-aspartame and β-AP in food from aspartame degradation.

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749 **Table 4:** Concentration of β -aspartame (β -APM) and β -aspartyl-L-phenylalanine (β -AP) in food
750 from aspartame degradation

Food	Conditions	% APM degraded	% degradation of APM to β -AP	% degradation of APM to β -APM	Reference
Carbonated Beverages APM initial: 0.5 g/l	pH 2.5 Time: 52 weeks T 20°C	75	9	3.4	UN11, 1986
	pH 2.5 Time: 39 weeks T 30°C	95	18.3	1.8	
	pH 4.4 Time: 38 weeks T 20°C	21	1	1.4	
	pH 4.4 Time: 52 weeks T 30°C	62	10.5	3.8	

751

752 2.6. Case of need and proposed uses

753 Aspartame (E 951) is authorised in the EU as a sweetener. The maximum permitted levels (MPLs)
754 range from 25 to 6000 mg/kg in foods, except for tabletop sweeteners for which it is authorised
755 *quantum satis*.

756 Table 5 summarises foods that are permitted to contain aspartame (E 951) and the corresponding
757 MPLs as set by Commission Regulation (EU) No 1129/2011¹⁴ on food additives for use in foodstuffs.

758 **Table 5:** MPLs of aspartame (E 951) in foods according to the Commission Regulation (EU) No
759 1129/2011

Category number	Foods	restrictions/exception	Maximum level (mg/L or mg/kg as appropriate)
1.4	Flavoured fermented milk products including heat-treated products	only energy-reduced products or with no added sugar	1000
3	Edible ices	only energy-reduced or with no added sugar	800
4.2.2	Fruit and vegetables in vinegar, oil, or brine	only sweet-sour preserves of fruit and vegetables	300
4.2.3	Canned or bottled fruit and vegetables	only fruit energy-reduced or with no added sugar	1000
4.2.4.1	Fruit and vegetable preparations excluding compote	only energy-reduced	1000
4.2.5.1	Extra jam and extra jelly as defined by Directive 2001/113/EEC	only energy-reduced jams jellies and marmalades	1000
4.2.5.2	Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EEC	only energy-reduced jams, jellies and marmalades	1000
4.2.5.3	Other similar fruit or vegetable spreads	only dried-fruit-based sandwich spreads, energy-reduced or with no added sugar	1000
5.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	only energy-reduced or with no added sugars	2000
5.2	Other confectionery including breath refreshing microsweets	only cocoa or dried fruit based, energy reduced or with no added sugar	2000

¹⁴ Commission regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) N°1333/2008 of the European Parliament and of the Council establishing a Union list of food additives. The Panel noted that the Commission Regulation (EC) No. 1129/2011 of 11 November 2011 will enter into force on June, 1st 2013 but confirm the approved uses of aspartame as a food additives as described in previous directive :

- Council Directive No. 94/35/EC of 30 June 1994 on sweeteners for use in foodstuffs,

Category number	Foods	restrictions/exception	Maximum level (mg/L or mg/kg as appropriate)
5.2	Other confectionery including breath refreshing microsweets	only cocoa, milk, dried fruit or fat based sandwich spreads, energy-reduced or with no added sugar	1000
5.2	Other confectionery including breath refreshing microsweets	only starch based confectionery energy reduced or with no added sugar	2000
5.2	Other confectionery including breath refreshing microsweets	only confectionery with no added sugar	1000
5.2	Other confectionery including breath refreshing microsweets	only breath-freshening micro-sweets, with no added sugar	6000
5.2	Other confectionery including breath refreshing microsweets	only strongly flavoured freshening throat pastilles with no added sugar	2000
5.3	Chewing gum	only with added sugars or polyols, as flavour enhancer	2500
5.3	Chewing gum	only with no added sugar	5500
5.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	only starch based confectionery energy reduced or with no added sugar	2000
5.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	only confectionery with no added sugar	1000
5.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	only cocoa or dried fruit based, energy reduced or with no added sugar	2000
5.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	only sauces	350
6.3	Breakfast cereals	only breakfast cereals with a fibre content of more than 15 %, and containing at least 20 % bran, energy reduced or with no added sugar	1000
7.2	Fine bakery wares	only essoblaten - wafer paper	1000
7.2	Fine bakery wares	only fine bakery products for special nutritional uses	1700
9.2.	Processed fish and fishery products including molluscs and crustaceans	only sweet-sour semi-preserves of fish and marinades of fish, crustaceans and molluscs	300
11.4.1	Table-top Sweeteners in liquid form		<i>quantum satis</i>
11.4.2	Table-top Sweeteners in powder form		<i>quantum satis</i>
11.4.3	Table-top Sweeteners in tablets		<i>quantum satis</i>
12.4	Mustard		350
12.5	Soups and broths	only energy-reduced soups	110
12.6	Sauces		350
12.7	Salads and savoury based sandwich spreads	only <i>Feinkostsalat</i>	350
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	Products in this category can also use additives that are allowed in the corresponding food counterparts categories	1000
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)		800
14.1.3	Fruit nectars as defined by Council Directive 2001/112/EC and vegetable nectars and similar products	only energy-reduced or with no added sugar	600
14.1.4	Flavoured drinks	only energy reduced or with no added sugar	600
14.2.1	Beer and malt beverages	only alcohol-free beer or with an alcohol content not exceeding 1.2 % vol; 'Bière de table/Tafelbier/Table beer' (original wort content less than 6 %) except for 'Obergäriges Einfachbier'; Beers with a minimum acidity of 30 milli-equivalents expressed as NaOH; Brown beers of the 'oud bruin' type	600
14.2.1	Beer and malt beverages	only energy-reduced beer	25
14.2.3	Cider and perry		600
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15 % of alcohol		600

Category number	Foods	restrictions/exception	Maximum level (mg/L or mg/kg as appropriate)
15.1	Potato-, cereal-, flour- or starch-based snacks		500
15.2	Processed nuts		500
16	Desserts excluding products covered in category 1, 3 and 4	only energy-reduced or with no added sugar	1000
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms excluding chewable forms		2000
17.2	Food supplements supplied in a liquid form		600
17.3	Food supplements supplied in a syrup-type or chewable form		5500

760

761 Considering the very specific foods in which aspartame is allowed and the restrictions within each
762 food groups, it was not possible to disaggregate the foods in the food consumption database used for
763 this estimate. Therefore, approximations have been made and are explained in detail later in this
764 section.

765 2.6.1. Actual level of use of aspartame

766 2.6.1.1. Reported use levels or data on analytical levels of aspartame (E 951)

767 Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used
768 at a lower level than the MPL. For those additives where no MPL is set and which are authorised as
769 *quantum satis*, information on actual use levels is required. In the framework of Regulation (EC) No
770 1333/2008 on food additives and of Regulation (EC) No 257/2010 regarding the re-evaluation of
771 approved food additives, EFSA issued a public call for scientific data on 1 June 2011 for aspartame
772 (E 951) including current use and use patterns (i.e. which food categories and subcategories,
773 proportion of food within categories/subcategories in which it is used, actual use levels (typical and
774 maximum use levels), especially for those uses which are only limited by *quantum satis*).

775 Following the EFSA call, data from industry and others sources were received:

- 776 • from FoodDrinkEurope (FDE, 2011): usage data for several food uses were provided by its
777 memberships,
- 778 • from the International Sweetener's Association (ISA) for table-top sweetener products in the
779 form of tablet and powder (ISA, 2011, 2012),
- 780 • from the International chewing gum association (ICGA, 2011): data on the uses of aspartame
781 as a sugar substitute in chewing gum and as flavour enhancer in chewing gum with added
782 sugars,
- 783 • from the Spanish association on sweets (ProDulce, 2012): data on the uses of aspartame in
784 sweets and flavoured drinks (only cocoa-based).
- 785 • from national authorities:
 - 786 ○ the Austrian Agency for Health and Food Safety (AGES),
 - 787 ○ the Dutch Food and Consumer Product Safety Authority (NVWA),
 - 788 ○ The Slovakian State Veterinary and Food Institute of Bratislava,

789 • Data from an Italian publication (Arcella *et al.*, 2004) were also made available to EFSA.

790 **2.6.1.2. Summarised data on reported use levels in foods from industries and other sources**

791 Table 6 provides data on the use levels of aspartame in foods as reported by industry and as analysed
792 by national agencies. This table also shows the levels used for the refined exposure assessment
793 identified by the Panel based on data for several food categories in finished products reported by
794 industry or analytical data from other sources (Member States, scientific literature).

795 In the EU regulation, tabletop sweeteners are allowed to contain aspartame at *quantum satis* level.
796 Industrial data provided for this food group are only coming from International Sweetener's
797 Association, which did not consider them as being representative for the European market because
798 these data were received from only two tabletop sweeteners companies. However, in order to take into
799 account this food group in the refined exposure assessment, the Panel decided to use available
800 information from the other sources (analytical and published data). Therefore, in place of *quantum*
801 *satis* (QS) data, a maximum estimated usage level of 500 000 mg/kg was used by the Panel in its
802 refined exposure assessment for tabletop sweeteners. This estimated usage level appears to be
803 consistent with the upper end of the range reported by industry (ISA) for the tablet form.

804 The Panel noted that FoodDrinkEurope's membership and ISA do not cover all manufacturers in a
805 particular sector or country and that the values reported for each individual food category are a general
806 output, i.e. a compilation of the individual contributions provided by their members. Due to the limited
807 representativeness of some of the data received for the European market, the Panel decided not to take
808 these data into account for use in the refined exposure assessment with the exception of data received
809 from the International chewing gum association (ICGA, 2011) on the uses of aspartame as a sugar
810 substitute in chewing gum and as flavour enhancer in chewing gum with added sugars.

811 **Table 6:** Summary of levels used in the refined exposure assessment

Matching Foodex Food codes	Food items	MPL (mg/kg or mg/L)	Use levels reported by industry (mg/kg or mg/L)					Analytical data reported by food agencies/literature range min-max in mg/kg or mg/L (number of samples)				Levels used for calculation (mg/kg or mg/L)	
			FDE		ISA	ICGA	Produlce	Comments from industry	Austria	The Netherlands	Slovakia		Italy
			typical	max	typical-max	typical-max	typical (range)						
1.4 - Flavoured fermented milk products including heat-treated products	Water-based flavoured drinks, energy-reduced or with no added sugar	1000	50-350	50-1000				Partly representative of the European market		54-132 (n=12)	93.5 (n=1)		1000
3 - Edible ices	Edible ices energy reduced or with no added sugar	800	40	50				Limited representation of the European market					800
4.2 - Processed fruit and vegetables	Energy-reduced jams, jellies and marmalades/energy-reduced fruit and vegetable preparation	1000	350-800	1000				Partly representative of the European market	107-695 (n=137)		74.5 (n=1)		1000
5.1 - Cocoa and Chocolate products as covered by Directive 2000/36/EC		2000					500-1000	No information on the representativeness, most probably only representative of Spanish products					2000
5.2.2 - Other confectionery without added sugar		1000	500-800	500-1000			100-1000	Partly representative of the European market	68.3 (n=7)	12 (n=4)	151.1-911.7 (n=6)		1000
5.3.1 - Chewing gum with added sugar		2500					600-1450	Maximum levels up to 7% of chewing-gums and 50% of chewing-gum contains aspartame as flavour enhancer at a typical level.					1450
5.3.2 - Chewing gum without added sugar		5500					3650-5420	Maximum levels for 4% of the products and up to 70% of sugar-free chewing gum contain aspartame at a typical level.	40-1747 (n=5)		560.8 (n=1)		5420

Matching Foodex Food codes	Food items	MPL (mg/kg or mg/L)	Use levels reported by industry (mg/kg or mg/L)					Analytical data reported by food agencies/literature range min-max in mg/kg or mg/L (number of samples)				Levels used for calculation (mg/kg or mg/L)	
			FDE		ISA	ICGA	Produce	Comments from industry	Austria	The Netherlands	Slovakia		Italy
			typical	max	typical-max	typical-max	typical (range)						
6.3 - Breakfast cereals		1000											1000
9.2 - Processed fish and fishery products including molluscs and crustaceans		300											300
11.4 - Table-top Sweeteners		QS			8700-36,000 (Powder form) 100,000-360,000 (tablet form)			Limited representation of the European market. Response from 2 table-top sweeteners	<0.25-238,600 * (n=19)	230,647 (n=1, tablet form)		0-500,000 (n=10) (tablet form)	500,000
12.4 - Mustard		350											350
12.5 - Soups and broths		110											110
12.6 - Sauces		350	200	200				Limited representation of the European market. Levels refer to ketchup	<4 (n=2)				350
12.7 - Salads and savoury based sandwich spreads	only Feinkostsalat	350							<20 (n=6)				350
13.2 - Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding 13.1.5)		1000	180	180				Limited representation of the European market					1000
13.3 - Dietary foods for weight control diets intended to replace total daily food intake or an individual meal		800											800
14.1.3 - Fruit nectars as defined by Council Directive 2001/112/EC and vegetable nectars and		600							<20 -71 (n=19)				75 ¹

Matching Foodex Food codes	Food items	MPL (mg/kg or mg/L)	Use levels reported by industry (mg/kg or mg/L)					Analytical data reported by food agencies/literature range min-max in mg/kg or mg/L (number of samples)				Levels used for calculation (mg/kg or mg/L)	
			FDE		ISA	ICGA	Produlce	Comments from industry	Austria	The Netherlands	Slovakia		Italy
			typical	max	typical-max	typical-max	typical (range)						
similar products													
14.1.4.2 - Flavoured drinks with sweeteners		600	100-350	600			110	FDE: Representative for the European market. 600 mg/L only apply in 1 product, rest of the applications reported usage levels below 430 mg/L ProDulce: refer to powder, only for cocoa-based soft drinks	<1-496 (n=272)	<3-470 (n=40)	3.5-2559.1 ** (n=60)		600
14.2 - Alcoholic beverages, including alcohol-free and low-alcohol counterparts	Drinks consisting of a mixture of a non-alcoholic drink and beer, cider, perry, spirits or wine. Spirits drinks containing less than 15% alcohol by volume	600	100-350	600				Partly representative of the European market	<1-104 (n=47)		7.4-25.6 (n=4)		600
15.1 - Potato-, cereal-, flour- or starch-based snacks		500											500
15.2 - Processed nuts		500											500
16 - Desserts excluding products covered in category 1, 3 and 4		1000											1000
17 - Food supplements as defined in Directive 2002/46/EC[5] excluding food supplements for infants and young children		5500							<20-1845.4 (n=3)	<20-2245 (n=17)			2245

812 ¹ rounded figure
813 *no clear information on the form of the tabletop sweeteners but the Panel assumes that values refer to tablet forms.
814 ** 2 values out of the 60 exceed the MPL of 600 mg/kg (one on 'herbal tea with honey, cinnamon and orange'; another on 'milk chocolate with added sweeteners')

815 Following rules defined by the Panel (Annex B.I), maximum values between use levels and analytical data were used for the refined exposure. Data from
816 industry could be considered as non-representative due to the comments provided. Analytical data were considered as representative when the number of data
817 reported was above or equal to 10 samples. When no data were suitable, MPLs were used to estimate the exposure.

818 **2.7. Information on existing authorisations and evaluations of aspartame, methanol and**
819 **DKP**

820 **2.7.1. Existing authorisations and evaluations of aspartame**

821 Aspartame has been authorized in the EU for use in foods and as a tabletop sweetener by several
822 Member States since the 1980s.

823 The SCF evaluated aspartame in 1984 (SCF, 1985) and in 1988 (SCF, 1989). The SCF established an
824 acceptable daily intake (ADI) of 40 mg/kg body weight (bw)/day. Further reviews of data on
825 aspartame were carried out by the SCF in 2002 (SCF, 2002). In the 2002 evaluation, the SCF
826 concluded that there was no evidence to suggest a need to revise the ADI previously established
827 for aspartame.

828 The European legislation harmonized its use in foodstuffs in 1994 through the European Parliament¹⁵
829 and Council Directive 94/35/EC, following safety evaluations by the SCF (SCF, 1985; SCF 1989).

830 Aspartame was evaluated by the Joint FAO/WHO Expert Committee on Food Additives on several
831 occasions (JECFA, 1975, 1976, 1978, 1980, 1981). JECFA established an ADI of 40 mg/kg bw/day
832 (1980, 1981).

833 In 1974, US FDA (US Food and Drug Administration) approved aspartame for restricted use in dry
834 foods in the United States. This was followed by its full approval for use as an artificial sweetener in
835 1981. The FDA has set the ADI for aspartame at 50 mg/kg bw.

836 In 1996, a report suggesting a connection between aspartame and an increase in the incidence of brain
837 tumours in the USA was published (Olney *et al.*, 1996). The conclusions of this epidemiological study
838 have been criticised by a number of scientists who questioned the methodology used and the
839 interpretation of the data (Levy and Heideker, 1996; Ross, 1998; Smith *et al.*, 1998; Linet *et al.*, 1999;
840 Seife, 1999). The SCF considered this report at its 107th meeting in June 1997 and concluded that the
841 data did not support the proposed biphasic increase in the incidence of brain tumours (SCF, 1997). The
842 issue has also been considered by the FDA and by the UK Committee on Carcinogenicity of
843 Chemicals in Food, Consumer Products and the Environment (COC, 1996). The FDA stated that
844 analysis of the National Cancer Institute database on cancer incidence in the USA did not support an
845 association between the use of aspartame and increased incidence of brain tumours (FDA, 1996). The
846 COC agreed that the findings provided no evidence for the proposed biphasic increase in the incidence
847 of either all brain tumours or selected tumour types in the USA during the 1980's and concluded that
848 the data published by Olney *et al.* (1996) did not raise any concerns about the use of aspartame in the
849 UK (COC, 1996).

850 In 2002, a review of aspartame safety was published by AFSSA (Agence française de sécurité
851 sanitaire des aliments). AFSSA (2002) concluded that aspartame was not genotoxic and that the
852 previously conducted carcinogenicity tests in rodents did not indicate a relationship between treatment
853 with aspartame and the appearance of brain tumours:

854 *'Taking into account all the studies that have been conducted, the frequency of spontaneous tumours*
855 *in laboratory rats, the types of tumours observed and the absence of a dose-response relationship, it*
856 *was concluded that aspartame had no carcinogenic potential on the brain in experimental animals*
857 *(FDA FR, 1981-1984; Koestner, 1984; Cornell et al., 1984; Flamm, 1997).'*

858 AFSSA concluded that the epidemiological study by Olney *et al.* (1996) did not provide any scientific
859 evidence of a relationship between exposure to aspartame and the appearance of brain tumours.
860 AFSSA (2002) also reported on the study by Gurney *et al.* (1997) who published the results of a case-

¹⁵ Directive 94/35/EC of the European Parliament and of the Council of 30 June 1994 on sweeteners for use in foodstuffs. OJ L 237, 10.9.1994, p. 3-12.

861 controlled study on the relationship between the consumption of aspartame and the frequency of brain
862 tumours. AFSSA agreed with the authors' conclusion that no relationship could be established
863 between the consumption of aspartame and the frequency of brain tumours.

864 Furthermore AFSSA noted that the epidemiological data from the cancer registers in France did not
865 permit a possible aspartame-brain tumour relationship to be clearly determined, but they did show that,
866 at the time, the sale of this food additive in France was not accompanied by an increase in the
867 frequency of brain tumours or increased mortality from this disease in the general population.

868 The SCF in its opinion of 2002 agreed with the AFSSA's evaluation of data on a relationship between
869 exposure to aspartame and brain tumours in humans. The SCF's conclusions in the opinion from 2002
870 were supported by the UK Food Standards Agency (FSA, 2002).

871 In 2006, at the request from the European Commission, the Scientific Panel on Food Additives,
872 Flavourings, Processing Aids and Materials in Contact with Food (AFC) assessed a long-term
873 carcinogenicity study in rats exposed to aspartame performed by European Ramazzini Foundation
874 (ERF) (Soffritti *et al.*, 2006). On the basis of all the evidence available from the Soffritti *et al.* (2006)
875 study, other recent studies and previous evaluations, the AFC Panel concluded that there was no
876 reason to revise the previously established ADI for aspartame of 40 mg/kg bw/day as established by
877 the SCF in 1984 (SCF, 2002; EFSA, 2006). The Committee on Carcinogenicity of Chemicals in Food
878 Consumer Products and the Environment (COC) also evaluated the Soffritti *et al.* (2006) study on
879 aspartame following the evaluation by EFSA (COC, 2006). In light of the limitations in the design of
880 this study and the use of animals with a high infection rate, the COC considered that no valid
881 conclusions could be drawn from this study. Therefore, the COC agreed that the Soffritti *et al.* (2006)
882 study did not indicate a need for a review of the ADI for aspartame (COC, 2006).

883 In 2009, following a request from the European Commission, the ANS Panel delivered a scientific
884 opinion on the results of a second long-term carcinogenicity study in rats starting with pre-natal
885 exposure to aspartame, performed by the ERF (Soffritti *et al.*, 2007). The ANS Panel concluded that
886 there was no indication of any genotoxic or carcinogenic potential of aspartame and no reason to
887 revise the previously established ADI for aspartame of 40 mg/kg bw/day (EFSA 2009a, 2009b).

888 In 2009 a series of meetings of National Experts nominated by the EU Member States was organized
889 with the support of the EFSA Advisory Forum to review the scientific literature on aspartame
890 including non-peer reviewed and anecdotal evidence that had become available since 2002. The report
891 of these meetings was published in 2010, and included a re-evaluation of the data on the potential
892 carcinogenicity (including cancer epidemiology), genotoxicity and neurotoxicity of aspartame (EFSA,
893 2010a). The conclusion by the National Expert meeting of the EFSA Advisory Forum was that there
894 was no new evidence that required a recommendation to EFSA that the previous opinions on
895 aspartame adopted by the AFC and ANS Panels and the SCF should be reconsidered.

896 In 2011 the ANS Panel and EFSA evaluated a new long-term carcinogenicity study in mice exposed to
897 aspartame from the 12th day of fetal life until death (Soffritti *et al.*, 2010) and a prospective cohort
898 study on the association between intakes of artificially sweetened soft drinks and preterm delivery
899 (Halldorsson *et al.*, 2010). The authors of the first study concluded that aspartame induced cancer in
900 the livers and lungs of male Swiss mice. EFSA and the ANS Panel observed that the hepatic and
901 pulmonary tumour incidences reported by Soffritti *et al.* (2010) all fell within their own historical
902 control ranges for spontaneous tumours and noted that Swiss mice are known to have a high
903 background incidence of spontaneous hepatic and pulmonary tumours. The overall conclusion by the
904 ANS Panel and EFSA was that the information available from the Soffritti *et al.* (2010) publication
905 did not give reason to reconsider the previous evaluations of aspartame (EFSA 2011a, 2011b). The
906 authors of the Halldorsson *et al.* (2010) study concluded that there was an association between intake
907 of artificially sweetened soft drinks and preterm delivery in the cohort; however, additional studies
908 were required to reject or confirm the association. The ANS Panel advised EFSA on the need for
909 epidemiological expertise to provide additional insights on the methodology and statistical aspects of

910 this study, taking into account confounding factors (EFSA, 2011a). EFSA concluded that there was no
911 evidence available to support a causal relationship between the consumption of artificially sweetened
912 soft drinks and preterm delivery and that additional studies were required to reject or confirm the
913 association (EFSA, 2011b). In 2011, the French Agency for Food, Environmental, and Occupational
914 Health and Safety (ANSES) also concluded that no causal relationship between the consumption of
915 artificially sweetened beverages and the risk of pre-term delivery was established, and, as the authors
916 of the Halldorsson study mentioned, there was a need to perform further studies to negate or confirm
917 the results (ANSES, 2011).

918 **2.7.2. Existing authorisations and evaluations of methanol**

919 In 1997, WHO (World Health Organization) stated that both methanol and its metabolite formate
920 occur naturally in humans and that normal background levels should not pose any risk to health and
921 consequently that levels of human exposure that do not result in levels of blood formate above
922 background levels could be considered to pose insignificant risk. Based on information from limited
923 studies in humans, WHO concluded that occupational exposure to current exposure limits (around 260
924 mg/m³) or single oral exposure to approximately 20 mg/kg bw would fall into this category.

925 In 2003, the Center for the Evaluation of Risks to Human Reproduction (CERHR) evaluated the
926 potential effects of methanol on human reproduction and development and concluded that there was
927 minimal concern for adverse developmental effects when humans are exposed to methanol levels that
928 result in low blood methanol concentrations, i.e., < 10 mg/l blood (NTP-CERHR, 2003). The CERHR
929 Panel also concluded that there was negligible concern for adverse male reproductive effects when
930 exposed to methanol levels that result in a low blood methanol level (< 10 mg/L blood) but there was
931 insufficient evidence to assess the effects of methanol on female reproduction.

932 The Subcommittee on the Classification of Carcinogenic Substances of the Dutch Expert Committee
933 on Occupational Exposure Safety (DECOS), a committee of the Health Council, concluded that
934 methanol cannot be classified with respect to its carcinogenicity (comparable with EU class 'not
935 classifiable') (Health Council of the Netherlands, 2010). The subcommittee was further of the opinion
936 that results from genotoxicity tests indicate that methanol is not likely to have a genotoxic potential.

937 In March 2011, the UK COT (UK Committee on Toxicity of Chemicals in Food, Consumer Products
938 and the Environment) issued a statement on effects of chronic dietary exposure to methanol. The COT
939 was asked to consider the effects of chronic oral methanol exposure in the light of consumer concerns
940 that methanol arising from the breakdown of the sweetener aspartame could be harmful (COT, 2011).
941 In its statement, the COT concluded that exposure to methanol at the levels found in the diet (dietary
942 methanol has been estimated to be up to 1 g/day), both naturally occurring and from currently
943 permitted levels of aspartame, would not be expected to result in adverse effects.

944 The US Environmental Protection Agency (EPA) recently conducted a toxicological review of
945 methanol for all non-cancer endpoints. The report is expected to be published by the end of 2013.

946 **2.7.3. Existing authorisations and evaluations of DKP**

947 JECFA and SCF established an ADI for DKP of 7.5 mg/kg bw/day (JECFA, 1980; SCF, 1989).
948 According to Commission Regulation (EU) No 231/2012, aspartame can contain DKP up to 1.5 %
949 (expressed on dry weight basis). In 1983, the US FDA established an ADI for DKP of 30 mg/kg
950 bw/day (FDA, 1983).

951 **2.8. Exposure assessment**

952 Anticipated exposures to aspartame from its use as a food additive and its related by-product
953 compounds, methanol, DKP, phenylalanine and aspartic acid, have been estimated in this section of
954 the opinion. β -aspartame exposure was not considered due to its low presence, as aspartame is not
955 degraded to this product.

956 The estimated exposures are based on the degradation process of aspartame as presented in Figure 2.
 957 Exposure calculations are based on the complete metabolism of aspartame to phenylalanine, aspartic
 958 acid and methanol. The calculation of exposure to DKP is based on the occurrence data received by
 959 EFSA and summarised in Table 3.

960 **2.8.1. Food consumption data used for exposure assessment**

961 In 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database)
 962 was built from existing national information on food consumption at a detailed level. Competent
 963 authorities in European countries provided EFSA with data on the level of food consumption by the
 964 individual consumer from the most recent national dietary survey in their country (cf. Guidance of
 965 EFSA ‘Use of the EFSA Comprehensive European Food Consumption Database in Exposure
 966 Assessment’ (EFSA, 2011d)).

967 The food consumption data gathered at EFSA were collected by different methodologies and thus
 968 direct country-to-country comparison should be made with caution.

969 Anticipated exposures to aspartame from its use as a food additive and its related by-product
 970 compounds (methanol, DKP, phenylalanine and aspartic acid) have been calculated (mean and 95th
 971 percentile of consumers only) using the food consumption data at the individual level (e.g. raw data on
 972 food consumption by the individual consumer).

973 High-level consumption was only calculated for those foods and population groups where the sample
 974 size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011d). The Panel
 975 estimated chronic exposure for the following population groups: toddlers, children, adolescents, adults
 976 and the elderly. Calculations were performed using individual body weights.

977 Thus, for the present assessment, food consumption data were available from 26 different dietary
 978 surveys carried out in 17 different European countries, as mentioned in Table 7.

979 **Table 7:** Population groups considered for the exposure estimates of aspartame (E 951)

Population	Age range	Countries with food consumption surveys covering more than one day
Toddlers	from 12 up to and including 35 months of age	Belgium, Bulgaria, Finland, Germany, Italy, Netherlands, Spain
Children ¹⁶	from 36 months up to and including 9 years of age	Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden
Adolescents	from 10 up to and including 17 years of age	Belgium, Cyprus, Czech Republic, Denmark, France, Germany, Italy, Latvia, Spain, Sweden
Adults	from 18 up to and including 64 years of age	Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Spain, Sweden, UK
The elderly ¹⁷	Older than 65 years	Belgium, Denmark, Finland, France, Germany, Hungary, Italy

980

981 Consumption records were codified according to the FoodEx classification system (EFSA, 2011d).
 982 Nomenclature from FoodEx classification system has been linked to the Food Classification System as
 983 presented in the Commission Regulation (EU) No 1129/2011, part D, to perform exposure estimates.

¹⁶ The terms ‘children’ and ‘the elderly’ correspond respectively to ‘other children’ and the merge of ‘elderly’ and ‘very elderly’ in the Guidance of EFSA on the ‘Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment’ (EFSA, 2011d).

984 In order to have an accurate selection of foods in which aspartame is authorised and thus an accurate
985 exposure estimate of aspartame, in addition to the FoodEx nomenclature, the original national food
986 names (i.e. original food descriptors as written in the national surveys) were used as much as possible.

987 **2.8.2. Exposure to aspartame from its use as a food additive**

988 Exposure to aspartame (E 951) from its use as a food additive has been calculated by using (1) MPLs
989 as listed in Table 5 and (2) data on reported use levels, or data reported on analytical levels as listed in
990 Table 6, both combined with national consumption data for the five population groups. Detail
991 summary of total estimated exposure (using MPLs and use levels) per age class and survey is
992 presented in Annex C.

993 No specific food consumption data for diabetics were available, therefore this sub-group of the
994 population was not considered in this exposure estimate. However, aspartame intakes by diabetics
995 have been estimated in several publications based on conservative dietary exposure to aspartame
996 (using individual consumption data from food diary method and MPLs). The exposure ranges from
997 1.2-5.3 mg/kg bw/day at the mean, to 1.9-16.6 mg/kg bw/day at the high level (Garnier-Sagne *et al.*,
998 2001, Magnusson *et al.*, 2007, Huvaere *et al.*, 2012).

999 Most of the food items in which aspartame is authorised are subject to limitations (Table 5), therefore,
1000 specific foods within the Comprehensive Database were selected. The Panel noted that its estimates
1001 should be considered as being conservative as it is assumed that all processed foods contain the
1002 sweetener aspartame (E 951) added at the MPL or the maximum reported use levels.

1003 However, due to the very detailed limitations laid down in the legislation, it was not always possible to
1004 match food categories in the food consumption database.

1005 - Fine bakery wares for special nutritional purposes are not defined by EU legislation. Some
1006 Member States have their own definition, such as bakery ware that is targeted at diabetics. As
1007 it is not the intention of the Commission to define this category under 'foods intended for
1008 particular nutritional uses' (PARNUTS), category 13, it is referred to under category 7.2, fine
1009 bakery ware in the food categorisation system¹⁷. Indeed, these products are not targeted at the
1010 general population; therefore, the Panel decided not to consider this category in the dietary
1011 exposure estimate for aspartame in the general population.

1012 - Essoblaten-wafer paper is a specific food item in the fine bakery ware category that is not
1013 present in the FoodEx nomenclature. Since its weight is very low and its consumption most
1014 probably marginal, this food was not taken into account.

1015 - Breakfast cereals with a fibre content of more than 15% and containing at least 20% bran,
1016 energy-reduced or with no added sugar are a very specific kind of breakfast cereals, not
1017 consumed by children and not described as such in the consumption data nomenclature.
1018 Therefore, this food was not taken into account in the present estimate.

1019 - The distinction between products with and without added sugar was only possible for the food
1020 groups: *flavoured drinks, confectionery and chewing gums*, although this was unclear for some
1021 surveys. This may result in an over/under-estimation depending on the reporting of these
1022 products in the surveys. Therefore, the whole food groups were taken into account for
1023 flavoured fermented milk products including heat-treated products, edible ices, soups and
1024 broths, fruit nectars, desserts, jams and other fruit and vegetable preparations. This may result
1025 in an over-estimation depending of the real consumption of these products within the food
1026 groups.

¹⁷ Part D (food categories) of the Commission Regulation (EU) N°1129/2011 of 11 November 2011.

1027 - The food group decorations, coatings, fillings are not described and available in the
 1028 nomenclature of the consumption database; thus, this food group could not be taken into
 1029 account. This resulted in a minor underestimation.

1030 - The distinction between the form of tabletop sweeteners (tablet, powder, liquid) was not
 1031 available in the EFSA Comprehensive Database. Therefore, these three food groups were
 1032 considered as a whole and the highest reported use level was taken into account. This could
 1033 result in a minor overestimation.

1034 - The same applies to the food supplements: no distinction between the forms of the food
 1035 supplements (liquid, solid, chewable forms) is possible within the FoodEx nomenclature,
 1036 therefore these three food groups were considered as a whole and the highest levels (MPLs
 1037 and reported use levels) were taken into account. This represents a minor overestimation.

1038 Table 8 summarises the estimated exposure to aspartame (E 951) from its use as a food additive in all
 1039 five population groups.

1040 **Table 8:** Summary of anticipated exposure to aspartame (E 951) from its use as a food additive
 1041 using MPLs and reported use levels or analytical data on use levels in five population groups (min-
 1042 max across the dietary surveys in mg/kg bw/day)

	Toddlers (12-35 months)	Children (3-9 years)	Adolescents (10-17 years)	Adults (18-64 years)	The elderly (>65 years)
Estimated exposure using MPLs					
• Mean	3.2-16.3	2.3-12.8	0.8-4.0	0.8-8.6	0.5-4.4
• High level ¹⁸	11.8-36.9	7.1-32.9	2.3-13.3	2.5-27.5	1.5-23.5
Estimated exposure using reported use levels or analytical data					
• Mean	1.6-16.3	1.8-12.6	0.8-4.0	0.7-8.5	0.4-4.4
• High level ¹⁹	7.5-36.0	6.3-32.4	2.3-13.2	2.4-27.5	1.4-23.5

1043

1044 Despite being presented as the estimated exposure based on MPLs, the reported use levels for tabletop
 1045 sweeteners were used for this exposure estimate. Indeed, tabletop sweeteners do not have MPLs but
 1046 their conditions of use are defined in the legislation at *Quantum Satis* (QS).

1047 The Panel noted that no major changes were seen in the anticipated exposure to aspartame using the
 1048 reported use levels in comparison to the use of MPLs due to a very small difference observed between
 1049 reported use levels and MPLs (Table 8).

¹⁸ 95th percentile consumers only

1050 **2.8.3. Main food categories contributing to exposure of aspartame using MPLs**

1051 **Table 9:** Main food categories contributing to the total anticipated mean dietary exposure to
1052 aspartame (E 951) (>5% of total exposure) using MPLs and number of surveys in which each food
1053 category is contributing over 5%.

	Toddlers	Children	Adolescents	Adults	The elderly
Food Categories	% contribution to total exposure (Number of surveys)				
1.4 - Flavoured fermented milk products including heat-treated products	15-72 (7)	13-52 (13)	8-47 (10)	6-16 (13)	6-33 (7)
5.1 - Cocoa and chocolate products as covered by Directive 2000/36/EC	10-17 (6)	8-35 (15)	8-52 (12)	5-25 (14)	12-22 (2)
3 - Edible ices	5-22 (4)	5-21 (14)	5-29 (11)	7-17 (6)	8 (2)
14.1.4 - Flavoured drinks with sweeteners	6-25 (2)	7-15 (8)	8-44 (6)	7-35 (11)	11 (1)
4.2 - Processed fruit and vegetables*	13 (2)	5-10 (9)	6-8 (9)	5-26 (11)	6-46 (7)
14.1.3 - Fruit nectars as defined by Council Directive 2001/112/EC and vegetable nectars and similar products	10-56 (2)	44 (1)		20-33 (2)	13 (1)
16 - Desserts excluding products covered in category 1, 3 and 4	6.5-19 (3)	5.1-17.6 (7)	7-14 (4)	7-10 (3)	7 (1)
11.4 - Table-top sweeteners**	-	6-14.7 (2)	9-16 (2)	5-55 (11)	36-66 (5)
12.5 - Soups and broths	-	14 (1)	13 (1)		5 (1)
12.7 - Salads and savoury based sandwich spreads	-	9-12 (2)	14 (1)	7-25 (2)	
14.2 - Alcoholic beverages, including alcohol-free and low-alcohol counterparts	-	-		6-7 (5)	
15.1 - Potato-, cereal-, flour- or starch- based snacks	7 (1)	5 (1)	6-11 (3)		
12.6 - Sauces	-	5.8-8.2 (2)	5-9 (6)	8 (1)	
17 - Food supplements					10 (1)

1054 * this category fruits and vegetables encompasses all the foods under the 4.2 category (Table 5): fruit and vegetables in
1055 vinegar, oil, or brine, canned or bottled fruit and vegetables, fruit and vegetable preparations excluding compote, extra
1056 jam and extra jelly as defined by Directive 2001/113/EEC, jam, jellies and marmalades and sweetened chestnut puree as
1057 defined by Directive 2001/113/EEC, other similar fruit or vegetable spreads.

1058 ** by default, reported use levels for tabletop sweeteners were used for this estimate in order to take them into account even
1059 if QS.

1060 **** Total number of surveys may be greater than total number of countries as listed in Table 7, as some countries submitted
1061 more than one survey for a specific age range.

1062 As expressed previously, the Panel noted that the exposure estimate calculated with reported use
1063 levels/analytical levels was comparable to that calculated with MPLs. For this reason, a separate table
1064 on the contributions of food groups to the total exposure calculated with the reported use levels and
1065 analytical levels is not presented.

1066 **2.8.4. Exposure to methanol**

1067 The Panel noted that exposure to methanol is not only from aspartame as a food additive and from
1068 natural sources through the diet where it is a normal constituent, but also to a large extent from
1069 endogenous sources (basal endogenous pathway, endogenously metabolised pectin).

1070 Methanol exposure from all sources is presented in Table 12. Detail summary of total estimated
1071 exposure to methanol from all sources per age class and survey is presented in Annex D.

1072 **2.8.4.1. Methanol from anticipated exposure to aspartame from its use as a food additive**

1073 Consumers may be exposed to methanol from consumption of aspartame containing products as
1074 methanol is released from aspartame in the gastrointestinal tract. Approximately 10% methanol is
1075 released by weight of aspartame. Based on the anticipated exposure estimates for aspartame (Table 5)
1076 using MPLs or reported use levels or analytical data the estimated exposure to methanol from all food
1077 and beverage applications of the sweetener for the five population groups would range from 0.05 to
1078 1.6 mg/kg bw/day at the mean and from 0.2 to 3.7 mg/kg bw/day at the high level (95th percentile
1079 consumers only) (Table 10).

1080 **Table 10:** Summary of anticipated exposure to methanol from anticipated exposure to aspartame (E
1081 951) as a food additive using MPLs and reported use levels or analytical data on use levels in five
1082 population groups (min-max across the dietary surveys in mg/kg bw/day)

	Toddlers (12-35 months)	Children (3-9 years)	Adolescents (10-17 years)	Adults (18-64 years)	The elderly (>65 years)
Estimated exposure using MPLs					
• Mean	0.3-1.6	0.2-1.3	0.1-0.4	0.1-0.9	0.05-0.4
• High level ¹⁹	1.2-3.7	0.7-3.3	0.2-1.3	0.3-2.7	0.2-2.4
Estimated exposure using reported use levels or analytical data					
• Mean	0.2-1.6	0.2-1.3	0.1-0.4	0.1-0.8	0.04-0.4
• High level ¹⁹	0.8-3.6	0.6-3.2	0.2-1.3	0.2-2.7	0.1-2.4

1083

1084 **2.8.4.2. Methanol from natural food occurrence**

1085 Methanol may be present *per se* or can be released from foods including fruits, fruit juices, vegetables,
1086 roasted coffee, honey and alcoholic beverages. In fruit juices, concentrations vary widely (1-640 mg
1087 methanol/L) with an average of 140 mg/L (WHO, 1997). More recent measurements have confirmed
1088 that juices from fruits such as apples, bananas, grapes and oranges may contain methanol at levels
1089 ranging from 8-148 mg/L (Wu *et al.*, 2007; Hou *et al.*, 2008; Hammerle *et al.*, 2011). In the case of
1090 tomatoes, levels of methanol up to 240 mg/L juice and 200 mg/kg diced and canned tomatoes have
1091 been reported (Hou *et al.*, 2008; Anthon and Barrett, 2010). Likewise, vegetable juices from e.g.
1092 alfalfa sprouts, carrots and spinach were found to contain methanol levels ranging from 53-194 mg/L
1093 (Hou *et al.*, 2008). The highly variable methanol content not only depends on the type of fruit and
1094 vegetable, i.e. methanol present before processing, but also on methanol released from pectin through
1095 the action of pectinesterase during processing and storage (Massiot *et al.*, 1997).

1096 Methanol is also found in alcoholic drinks. It is a residue of manufacturing process of alcoholic
1097 beverages and maximum levels of methanol have been defined for spirit drinks in the Regulation (EC)
1098 No 110/2008. WHO reported methanol concentrations of 6-27 mg/L in beer, 96-321 mg/L in wine and
1099 10-220 mg/L in distilled spirits (WHO, 1997). Depending on the cultivars, apple ciders have been
1100 reported to contain 300-700 mg methanol/L, whereas a methanol content of up to 1.6 g/L has been
1101 reported in apple spirit (Hang and Woodams, 2010).

¹⁹ 95th percentile consumers only

1102 To estimate exposure to methanol due to its natural presence in foods, the Panel used data from
 1103 Magnuson *et al.* (2007) and data from (2012), a TNO database compiled from 340 literature references
 1104 for methanol in foods from papers dating from 1965 to 2006. The Panel noted that occurrence of
 1105 methanol predominantly in fruit and fruit-based beverages is consistent and in line with current
 1106 knowledge (Annex B-II) and that methanol levels for individual products vary considerably.

1107 In its review, the Panel considered that quantitative measurements of methanol in foods were generally
 1108 reliable and that data generated using what would now be considered out-dated methodologies could
 1109 be considered reasonably accurate (the current analytical method used for the determination of
 1110 methanol in foods is based on headspace GC/MS).

1111 Exposure to methanol has been estimated by the Panel using the raw individual food consumption data
 1112 at the most detailed level from the Comprehensive Database (EFSA, 2011d) and using the mean
 1113 methanol concentration, calculated from the natural occurrence data range reported in Annex B-II.
 1114 Mean values have been calculated by the Panel for the purpose of estimating background chronic
 1115 intake of methanol from natural food occurrences which were based on the assumption that the
 1116 distribution of composition data per category (only when range is expressed) followed a uniform
 1117 distribution.

1118 Considering all the dietary surveys and populations groups, the exposure estimates for methanol from
 1119 natural food occurrence range from 0.2-1.4 mg/kg bw/day at the mean, to 0.6-3.8 mg/kg bw/day for
 1120 high level consumers (Table 11).

1121 **Table 11:** Summary of anticipated exposure to methanol from natural food occurrence in five
 1122 population groups (min-max across the dietary surveys in mg/kg bw/day)

	Toddlers (12-35 months)	Children (3-9 years)	Adolescents (10-17 years)	Adults (18-64 years)	The elderly (>65 years)
Estimated exposure					
• Mean	0.2-1.4	0.3-1.4	0.2-0.6	0.3-0.6	0.2-0.6
• High level ²⁰	0.9-3.8	0.9-3.3	0.6-1.4	0.7-1.6	0.6-1.4

1123

1124 Mean and 95th percentile total intakes of methanol from foods naturally containing methanol have
 1125 been reported to be 10.7 and 33.3 mg methanol/kg bw/day for US consumers among the general
 1126 population using Daily Intake via Natural Food Occurrence (DINFO) analysis (Magnuson *et al.*,
 1127 2007). However, this analysis did not include intake from processed foods or from food sources such
 1128 as potatoes or onions, or the methanol released by pectin breakdown in fruits and vegetables during
 1129 processing and storage, and as such will be an underestimation of the true dietary exposure to
 1130 methanol.

1131 The Panel noted that exposure estimates for methanol from natural sources reported in the above
 1132 mentioned literature though lower, were globally within the same order of magnitude as the estimates
 1133 derived by the Panel (mean and high consumers assuming 70 kg body weight would give a range of
 1134 0.2 to 0.5 mg/kg bw/day, respectively, in comparison to 0.2-1.6 mg/kg bw/day in adults population).

1135 **2.8.4.3. Methanol from endogenous pathways**

1136 In addition to the direct contribution of methanol from fruits and vegetables and their juices, there is
 1137 clear evidence for the production of methanol in the gastrointestinal tract from ingested pectins and
 1138 endogenous production in other parts of the body, such as liver and brain (WHO, 1997).

²⁰ 95th percentile of consumers only

1139 In two papers (Taucher *et al.*, 1995; Lindinger *et al.*, 1997), methanol concentration was measured in
1140 the breath of human volunteers using proton-transfer-reaction mass spectrometry in a selected ion flow
1141 drift tube. The volunteers were male, in a fasted state to minimize the contribution of dietary pectin to
1142 the measured methanol and had received ethanol at time zero and at 2.5h in order to maintain blood
1143 ethanol concentration in excess of 150 mg/L. The latter approach was used to competitively inhibit
1144 methanol metabolism by alcohol dehydrogenase and CYP2E1, thereby enabling the measurement of
1145 methanol production by the body. The authors showed that basal endogenous methanol production by
1146 the human body, measured over a period of 6-12 hours, amounted to between 300 and 600 mg
1147 methanol/day (Lindinger *et al.*, 1997). The same authors showed that the ingestion of 10 g to 15 g of
1148 pectin (corresponding to 1 kg apples) resulted in the additional production of 400-1400 mg
1149 methanol/person/day.

1150 Dhareshwar and Stella (2008) estimated that exposure to methanol from combined endogenous and
1151 exogenous sources was between 0.13 and 1.03 g/day.

1152 In another study performed in six healthy male subjects, basal endogenous methanol production was
1153 assessed over a period of 5h. The subjects were given an infusion of ethanol to inhibit methanol
1154 metabolism after a period of fasting and abstinence from alcohol (Haffner *et al.*, 1998). Methanol was
1155 measured in blood using gas chromatography. The observed rate of increase in blood methanol
1156 concentration was linear between 30 min and 5h and ranged from 0.08 – 0.35 mg/L/hour. The Panel
1157 noted that when converting the blood levels to whole body production using a volume of distribution
1158 (V_D) for methanol of 0.77 L/kg (Graw *et al.*, 2000), the daily endogenous production of methanol
1159 could be approximated to 1.5-6.5 mg/kg bw/day, or 107-453 mg/person/day.

1160 Basal endogenous methanol production was measured in 22 female and 13 male volunteers given 4-
1161 methylpyrazole to inhibit ADH in a cross-over study (Sarkola and Eriksson, 2001). Compared to the
1162 placebo control, a linear increase in blood methanol levels up to 3.6 μ M/h after 4-methylpyrazole
1163 application was measured. The Panel noted that when converting the blood levels to whole body
1164 production using a volume of distribution (V_D) for methanol of 0.77 L/kg ((Graw *et al.*, 2000), a daily
1165 endogenous production of methanol could be estimated to be 2.1 mg/kg bw/day or 149 mg/person/day.

1166 Ernstgård *et al.* (2005) investigated uptake and disposition of inhaled methanol in four female and four
1167 male subjects who had not consumed any alcohol, drugs or fruits for at least 48h prior to the beginning
1168 of the measurements. From the absorption, half-life and area under the concentration-time curve
1169 (AUC) values reported by the authors for methanol, the Panel derived a value for endogenous
1170 production of 9.1 mg/kg bw/day or 640 mg methanol/person/day.

1171 The Panel concluded that basal endogenous methanol production ranges from 2 to 9 mg/kg bw/day
1172 (minimum to maximum) in adults. In the elderly, the endogenous methanol production may be up to
1173 15% less as the resting metabolic rate is decreasing with age (Speakman and Westerterp, 2010). In
1174 infants and children less than 18 years of age there is a higher metabolic rate per body mass as
1175 compared to the adult (Wang, 2012). Hence, use of the endogenous production rate of the adult
1176 (expressed in mg/kg) in lieu of experimentally measured methanol production rates in toddlers may
1177 underestimate the endogenous production rate in this age by a factor of two. In addition, the body
1178 increases methanol production following consumption of pectin-containing foods such as apples by an
1179 additional 6-20 mg/kg bw/day (Lindinger *et al.*, 1997).

1180 To estimate exposure to methanol due to pectin degradation, the Panel used data on pectin content
1181 from Baker (1997), Mahattanotawee *et al.* (2006) and Holloway *et al.* (1983).

1182 The exposure to methanol from pectins has been estimated by the Panel using the raw individual food
1183 consumption data at the most detailed level from the Comprehensive Database (EFSA, 2011d) and
1184 using the occurrence levels of methanol derived from pectin contents reported. Values are detailed in
1185 Annex B-III. The Panel noted that occurrence of pectin is predominantly in fruit and vegetables. The

1186 degradation factor from pectin to methanol was derived from Lindinger *et al.* (1997) i.e. 10 g pectins
1187 resulted in a mean additional production of methanol of 900 mg.

1188 **2.8.4.4. Combined exposure to methanol from endogenous and exogenous sources**

1189 The Panel estimated the anticipated combined exposure to methanol from endogenous sources (basal
1190 endogenous pathway, endogenously metabolised pectin) and exogenous sources (aspartame as a food
1191 additive and natural food occurrence). A summary of the estimated exposures is given in Table 12.

1192 The exposure to methanol has been estimated by the Panel using the raw individual food consumption
1193 data at the most detailed level from the Comprehensive Database (EFSA, 2011d) and using the
1194 occurrence data (Table 5 and Annexes B-II; B-III).

1195 The Panel estimated that the exposure to methanol from all sources (basal endogenous pathway and
1196 endogenously metabolised pectin, natural food occurrence through the diet and aspartame as a food
1197 additive) in five population groups would range from 8.4 to 18.9 mg/kg bw/day at the mean and from
1198 15.1 to 35.1 mg/kg bw/day for high-level consumers.

1199

1200 **Table 12:** Total estimated exposure of methanol from all sources (food additive, endogenous pathways and natural food occurrence) in five population
1201 groups (min-max across the dietary surveys in mg/kg bw/day)

	Toddlers		Children		Adolescents		Adults		The elderly	
	Mean	High level	Mean	High level	Mean	High level	Mean	High level	Mean	High level
	mg/kg bw/day									
Exposure range of methanol from basal endogenous pathway	5.5 ^a	9	5.5	9	5.5	9	5.5	9	5.5	9
Exposure range of methanol from endogenously metabolised pectin	7.9-12.5	15.2-24.1	3.9-10.5	7.7-20.0	2.1-5.0	5.3-10.5	2.4-4.2	5.1-7.9	3.0-4.5	6.0-8.1
Exposure range of methanol from natural food occurrence	0.2-1.4	0.9-3.8	0.3-1.4	0.9-3.3	0.2-0.6	0.6-1.4	0.3-0.6	0.7-1.6	0.2-0.6	0.6-1.4
Exposure range of methanol from all endogenous pathways (basal, endogenously and metabolised pectin) and natural food occurrence	13.8-18.4	9.0-33.1	9.8-17.4	20.3-36.8	8.0-10.7	14.3-19.5	8.2-10.3	14.1-16.9	8.9-10.6	15.0-17.1
Exposure range of methanol from aspartame as a food additive (using MPLs)	0.3-1.6	1.2-3.7	0.2-1.3	0.7-3.3	0.1-0.4	0.2-1.3	0.1-0.9	0.3-2.7	0.05-0.4	0.2-2.4
Total anticipated exposure to methanol from all sources (endogenous pathways (basal, endogenously metabolised pectin) and natural food occurrence and aspartame as a food additive)	14.7-18.9	26.3-35.1	10.4-17.6	18.1-31.8	8.4-11.0	15.5-20.2	8.4-10.3	15.1-18.2	9.0-10.7	15.8-17.9

1202 ^a = average of 2-9 mg/kg bw/day (assuming uniform distribution)

1203 The contribution of aspartame-derived methanol is shown in Table 13.

1204 **Table 13:** Total contribution of aspartame derived-methanol to the exposure from all sources
 1205 (aspartame as a food additive, endogenous (basal and pectin-derived) and natural food occurrence) in
 1206 five population groups (min-max in %)

	Toddlers	Children	Adolescents	Adults	The elderly
	Mean	Mean	Mean	Mean	Mean
	%				
Contribution of aspartame-derived methanol (using MPLs) for average consumers of aspartame to total mean anticipated exposure to methanol from all sources	1.7-9.2	1.6-9.7	0.8-4.8	0.7-9.4	0.5-4.7
Contribution of methanol from endogenous source (basal + pectin derived) to total mean anticipated exposure to methanol from all sources	84-95	86-94	91-96	87-96	91-97
Contribution of methanol from natural food occurrence to total mean anticipated exposure to methanol from all sources	1.2-7.6	2.1-7.8	1.9-5.5	2.7-6.2	2.4-5.3

1207

1208 The Panel noted that for average consumers of aspartame, the contribution from aspartame to the
 1209 overall exposure to methanol ranged from 1% to 10% in the general population.

1210 In this estimate, the Panel also noted that exposure to methanol from natural food occurrence is a
 1211 minor contributing source (< 10%) compared to exposure from endogenous pathways (> 80% in all
 1212 population groups), and also that the exposure from aspartame-derived methanol is similar to methanol
 1213 exposure from natural food occurrence.

1214 In addition to aspartame, there are other food additives that release methanol as a result of their
 1215 metabolism. Examples of methanol releasing food additives include dimethyldicarbonate (E 242,
 1216 DMDC), which is used in beverages and breaks down to CO₂ and methanol; pectins (E 440) that are
 1217 subject to the methyl ester linkage cleavage in the upper gastrointestinal tract as described above;
 1218 methyl p-hydroxybenzoate and sodium methyl p-hydroxybenzoate (E218, E219); hexamethylene
 1219 tetramine (E239). Therefore, while not considering the methanol release from other food additives the
 1220 present estimation is considered as being an under-estimation of the total exposure to methanol.

1221 An additional source of exposure to methanol is from extraction solvents. The Panel noted that in the
 1222 EU the maximum residue limits for methanol in extracted foodstuff or food ingredients is set at 10
 1223 mg/kg.²¹

1224 **2.8.5. Exposure to 5-benzyl-3,6-dioxo-2-piperazine acetic acid (DKP) from anticipated**
 1225 **exposure to aspartame from its source as a food additive**

1226 5-Benzyl-3,6-dioxo-2-piperazine acetic acid (DKP) exposure intakes were estimated from anticipated
 1227 exposure to aspartame (E 951) from its use as a food additive. DKP is a degradation product of
 1228 aspartame. The degradation from aspartame to DKP ranges from 1.5% (EU specifications) up to 24%
 1229 based on the analytical data reviewed by the Panel and reported in Table 3. The maximum of 24% is
 1230 for soft drinks and is estimated from an average degradation of 80% of aspartame of which DKP
 1231 accounts for 30%. The concentrations of DKP in foods taken into account by the Panel in estimating
 1232 exposure were assigned to the relevant food categories in which aspartame is authorised according to
 1233 MPLs and use levels scenarios as described in Annex B-IV. For foods categories where data were

²¹ Directive 2009/32/EC of the European Parliament and of the Council of 23 April 2009

1234 available, the highest value was taken into account; for the ones where no degradation percentage of
1235 DKP was available, the highest value (24%) from the whole database was used. In a few food
1236 categories (processed fish and fishery products, tabletop sweeteners, food supplements) depending on
1237 the food type (solid vs. liquid), other degradation percentages of DKP were used.

1238 Anticipated exposures to DKP from aspartame consumption are described in the Table 14. Detail
1239 summary of total estimated exposure (from the use of aspartame MPLs) per age class and survey is
1240 presented in Annex E.

1241 **Table 14:** Summary of anticipated exposure to DKP from anticipated exposure to aspartame (E 951)
1242 as a food additives using MPLs and reported use levels or analytical data on use levels in five
1243 population groups (min-max across the dietary surveys in mg/kg bw/day)

	Toddlers (12-35 months)	Children (3-9 years)	Adolescents (10-17 years)	Adults (18-64 years)	The elderly (>65 years)
Estimated exposure using MPLs					
• Mean	0.3-1.9	0.2-1.3	0.1-0.7	0.1-1.6	0.1-0.8
• High level ²²	1.2-4.1	0.5-3.2	0.3-2.5	0.3-5.5	0.2-4.8
Estimated exposure using reported use levels or analytical data					
• Mean	0.2-1.9	0.2-1.3	0.1-0.7	0.1-1.6	0.1-0.8
• High level ²²	0.7-3.9	0.5-3.1	0.3-2.5	0.3-5.5	0.2-4.8

1244

1245 Based on the anticipated exposure estimates for aspartame using MPLs (Table 5), the estimated
1246 exposure to DKP from all food and beverage use of the sweetener would approximately, 0.1 to 1.9
1247 mg/kg bw/day at the mean and 0.2 to 5.5 mg/kg bw/day for the 95th percentile consumer across the
1248 dietary surveys and populations groups.

1249 **2.8.6. Exposure to phenylalanine**

1250 The Panel noted that exposure to phenylalanine is not only from aspartame as a food additive but also
1251 largely from natural food occurrence through the diet.

1252 Phenylalanine exposure from both sources is presented in Table 16. Detail summary of total estimated
1253 exposure (from the use of aspartame MPLs) per age class and survey is presented in Annex E.

1254 **2.8.6.1. Phenylalanine from natural food occurrence**

1255 Phenylalanine is an essential amino acid and therefore must be supplied by the diet. The recommended
1256 aromatic amino acid requirement (phenylalanine or tyrosine) is set at 25 mg/kg bw/day (WHO, 2007).
1257 In the US, the Institute of Medicine sets estimated average requirement (EAR) for amino acids;
1258 phenylalanine + tyrosine EAR for adults are estimated at 27 mg/kg bw/day (IOM, 2005). The mean
1259 daily consumption of phenylalanine is estimated in the US at 3.4 g/day (IOM, 2005). A French study
1260 (Rousseau *et al.*, 2006) reported in an AFSSA report on protein intake (AFSSA, 2007) estimated the
1261 daily intakes of different populations: from sedentary people to sportspersons (several levels of
1262 expenditure of physical energy). The mean phenylalanine intake for sedentary people was estimated to
1263 be 3.8 g/day.

1264 Additional information on phenylalanine intake from natural food occurrence through the diet and
1265 phenylalanine content per food category of the EFSA Comprehensive Database was provided to EFSA

²² 95th percentile of consumers only

1266 (König *et al.*, (2012) unpublished data). Concentration data of phenylalanine in foods came from the
 1267 German food composition database (Bundeslebensmittelschlüssel). The levels of phenylalanine
 1268 content per food category (according to level 2 of the food additives nomenclature from Regulation
 1269 No 1129/2011) range from 0.01 mg/g in wine to 31.05 mg/g in meat imitates; some food groups do not
 1270 contain any phenylalanine. These levels linked to the EFSA Comprehensive Database allow
 1271 calculation of phenylalanine intake from the diet. These intakes range from 0.9 g/day (corresponding
 1272 to 93.0 mg/kg bw/day for toddlers) up to 4.1 g/day (corresponding to 58.7 mg/kg bw/day for adults) at
 1273 the mean within the EU population (see Table 16).

1274 **2.8.6.2. Phenylalanine from anticipated exposure to aspartame from its source as a food**
 1275 **additive**

1276 Phenylalanine exposure intakes were estimated from anticipated exposure to aspartame (E 951) from
 1277 its use as a food additive. Aspartame is fully hydrolysed in the gastrointestinal tract to L-
 1278 phenylalanine, L-aspartic acid and methanol. Taking the molecular weight of phenylalanine into
 1279 account, the factor for aspartame exposure to phenylalanine exposure is 56%. Phenylalanine intakes
 1280 from aspartame consumption are described in Table 15.

1281 **Table 15:** Summary of anticipated exposure to phenylalanine from estimated exposure to aspartame
 1282 (E 951) as a food additive using MPLs and reported use levels or analytical data on use levels in five
 1283 population groups (min-max across the dietary surveys in mg/kg bw/day)

	Toddlers (12-35 months)	Children (3-9 years)	Adolescents (10-17 years)	Adults (18-64 years)	The elderly (>65 years)
Estimated exposure using MPLs					
• Mean	1.8-9.2	1.3-7.2	0.4-2.3	0.4-4.8	0.3-2.5
• High level ²³	6.6-20.7	4.0-18.4	1.3-7.5	1.4-15.4	0.9-13.2
Estimated exposure using reported use levels or analytical data					
• Mean	0.9-9.2	1.0-7.1	0.4-2.3	0.4-4.8	0.2-2.5
• High level ²³	4.2-20.2	3.5-18.2	1.3-7.4	1.3-15.4	0.8-13.2

1284

1285 **2.8.6.3. Combined exposure to phenylalanine from the use of aspartame as a food additive and**
 1286 **from natural food occurrence through the diet**

1287 In this estimate, the Panel noted that exposure to phenylalanine from aspartame-derived phenylalanine
 1288 for all population groups ranged from 1% to 8% at the mean (compared to the total intake of
 1289 phenylalanine from aspartame and natural food occurrence).

1290 **Table 16:** Summary of anticipated combined exposure to phenylalanine from exposure to aspartame
 1291 (E 951) as a food additive using MPLs and from natural food occurrence in five population groups
 1292 (min-max across the dietary surveys in mg/kg bw/day)

	Toddlers (12-35 months)	Children (3-9 years)	Adolescents (10-17 years)	Adults (18-64 years)	The elderly (>65 years)
Estimated exposure from natural food occurrence					
• Mean	93.0-167.1	72.2-136.7	39.1-79.0	35.3-58.7	32.5-41.6
• High level ²³	144.2-322.2	126.5-214.3	73.1-130.3	60.2-88.7	54.6-64.4

²³ 95th percentile of consumers only

Estimated exposure using MPLs					
• Mean	1.8-9.2	1.3-7.2	0.4-2.3	0.4-4.8	0.3-2.5
• High level ²³	6.6-20.7	4.0-18.4	1.3-7.5	1.4-15.4	0.9-13.2
Combined exposure to PHE					
• Mean	95.4-176.3	74.0-140.5	41.3-80.7	37.9-59.1	34.8-41.9
• High level ²³	146.5-331.1	130.9-224.1	78.4-136.9	64.8-89.1	58.9-68.2

1293

1294 **2.8.7. Exposure to aspartic acid from anticipated exposure to aspartame from its source as a**
1295 **food additive**

1296 Aspartic acid exposure intakes were estimated from anticipated exposure to aspartame (E 951) from its
1297 use as a food additive. Taking the molecular weight into account, the factor to derive aspartic acid
1298 exposure from aspartame exposure is 45%. Aspartic acid intakes from aspartame consumption are
1299 described in the Table 17. Detail summary of total estimated exposure (from the use of aspartame
1300 MPLs) per age class and survey is presented in Annex E.

1301 **Table 17:** Summary of anticipated exposure to aspartic acid from estimated exposure to aspartame
1302 (E 951) as a food additives using MPLs and reported use levels or analytical data on use levels in five
1303 population groups (min-max across the dietary surveys in mg/kg bw/day)

	Toddlers (12-35 months)	Children (3-9 years)	Adolescents (10-17 years)	Adults (18-64 years)	The elderly (>65 years)
Estimated exposure using MPLs					
• Mean	1.5-7.4	1.0-5.8	0.4-1.8	0.3-3.9	0.2-2.0
• High level ²³	5.3-16.7	3.2-14.9	1.0-6.0	1.1-12.4	0.7-10.6
Estimated exposure using reported use levels or analytical data					
• Mean	0.7-7.4	0.8-5.7	0.4-1.8	0.3-3.8	0.2-2.0
• High level ²³	3.4-16.3	2.8-14.7	1.0-6.0	1.1-12.4	0.6-10.6

1304

1305 In the US, the Third National Health and Nutrition Examination Survey (1988-1994) estimated the
1306 mean intake of aspartic acid of 6.5 g/day (IOM, 2005). High levels (99th percentile) were estimated for
1307 the 31-50 year old male population at 15.4 g/day.

1308 **2.9. Uncertainty analysis**

1309 According to the guidance provided by the EFSA opinion (EFSA, 2006), the following sources of
1310 uncertainties have been considered. These have been presented and discussed in detail in the sections
1311 above:

1312 - **Input data:**

1313 • Food consumption data: the data used for the exposure estimates were data reported at the
1314 individual level in several different dietary surveys that applied different methodologies.
1315 Under-reporting and/or misreporting often represent a bias in dietary surveys.

1316 • Food nomenclature: the food nomenclatures used in the dietary surveys considered and
1317 then the FoodEx nomenclature used in the Comprehensive database are different from the

1318 Food Classification System developed for the definition of food additives uses in the
 1319 Commission Regulation (EU) No 1129/2011. Therefore, linking between different food
 1320 nomenclatures leads to uncertainties.

- 1321 • Reported use levels: the estimates calculated are considered as being conservative because
- 1322 they are based on the assumption that all products within a given food category contain
- 1323 aspartame at the maximum reported use level.

1324 **Table 18:** Qualitative evaluation of influence of uncertainties

Sources of uncertainties	Direction ^(a)
Consumption data: different methodologies / representativeness / under reporting / misreporting / no portion size standard	+/-
Extrapolation from food consumption survey of few days to estimate chronic exposure	+
Linkage between reported use levels and food items in the consumption database: uncertainties on which precise types of food the use levels refer.	+/-
Occurrence data: maximum reported use levels within a food category	+
Exposure model: uncertainty in possible national differences in use levels of food categories, data set not fully representative of foods on the EU market, exposure calculations based on the maximum reported use levels (no use of typical use levels when available)	+

1325 (a) +: uncertainty with potential to cause over-estimation of exposure;
 1326 -: uncertainty with potential to cause under-estimation of exposure.
 1327

1328 **3. Biological and toxicological data of aspartame**

1329 A public call was launched by EFSA in 2011 following which original reports of unpublished studies
 1330 were received (<http://www.efsa.europa.eu/en/dataclosed/call/110601.htm>). The Panel was aware that
 1331 many of these reports would have been available at the time of the JECFA and SCF evaluations. The
 1332 present evaluation by the Panel of the biological and toxicological data includes the assessment of all
 1333 these unpublished studies, together with the published literature identified until the end of November
 1334 2012.

1335 The Panel noted that the majority of these unpublished studies were performed in the period 1970-
 1336 1978 and as such had not been performed according to Good Laboratory Practice (GLP) and
 1337 Organisation for Economic Co-operation and Development (OECD) guidelines
 1338 ([http://www.oecd.org/chemicalsafety/testingofchemicals/oecdseriesonprinciplesofgoodlaboratorypract](http://www.oecd.org/chemicalsafety/testingofchemicals/oecdseriesonprinciplesofgoodlaboratorypracticeglpandcompliancemonitoring.htm)
 1339 [iceglpandcompliancemonitoring.htm](http://www.oecd.org/chemicalsafety/testingofchemicals/oecdseriesonprinciplesofgoodlaboratorypract)). However, according to the Panel, the fact that the studies were
 1340 old and were not performed according to current standards should not *per se* disqualify them from
 1341 being included in the risk assessment of aspartame. As long as the design of any such study and the
 1342 reporting of the data were appropriate, the Panel agreed that the study should be considered in the re-
 1343 evaluation of the sweetener.

1344 **3.1. Absorption, distribution, metabolism and excretion of aspartame**

1345 Throughout the following sections, the term aspartame refers to the α -form.

1346 **3.1.1. Studies on absorption, distribution, metabolism and excretion received following a**
 1347 **public call for data which were available at the time of the previous JECFA and SCF**
 1348 **evaluations**

1349 The Panel noted that although advances in analytical methodologies have occurred since, the studies
 1350 were conducted in the 1970s using methods that were available and appropriate at that time. The Panel
 1351 considered the results suitable for assessment despite the methodological limitations of these studies.

1352 In various animal species, including mouse, rat, rabbit, dog and monkey, the absorption, distribution,
1353 metabolism and excretion of aspartame have been investigated using different radiolabelled forms of
1354 ¹⁴C- aspartame (E15, 1972; E17, 1972; E18, 1972; E80, 1974). Radiolabelling was located either on
1355 the phenylalanine or aspartic acid moiety, or on the methyl group. In some studies (namely in rats and
1356 dogs) data have been compared with corresponding animals receiving ¹⁴C(U)-phenylalanine, ¹⁴C(U)-
1357 aspartic acid or ¹⁴C-methanol.

1358 In mice given 20 mg/kg bw of [¹⁴C(U)-phe]-aspartame by gavage, the radioactivity found in plasma,
1359 urine, faeces and expired air clearly demonstrated that aspartame was hydrolysed in the gut of the
1360 mouse before absorption of its phenylalanine moiety occurred (E18, 1972). In plasma, the major
1361 fractions of ¹⁴C-materials corresponded to phenylalanine or tyrosine, both of which were incorporated
1362 in plasma proteins.

1363 Several studies have been carried out in rats, primates and man receiving oral ¹⁴C-aspartame (20-30
1364 mg/kg bw) radiolabelled at the phenylalanine or aspartic acid moiety or on the methyl group (E15,
1365 1972). Comparisons were made with rats given ¹⁴C(U)-phenylalanine, ¹⁴C(U)-aspartic acid, ¹⁴C-
1366 methylphenylalanine or ¹⁴C-methanol. In all studies, results clearly indicated that aspartame was
1367 hydrolysed in the gut.

1368 When aspartame was radiolabelled at the phenylalanine moiety, ([¹⁴C(U)-phe]-aspartame), it was
1369 handled in a fashion similar to that of the naturally occurring amino acid phenylalanine.

1370 When aspartame was radiolabelled at the aspartic acid moiety, [¹⁴C(U)-asp]-aspartame, the compound
1371 was metabolised by experimental animals to free ¹⁴C-L-aspartic acid, which was subsequently
1372 metabolised in the same way as aspartic acid in the diet. The major fraction (60-70% of the dose) of
1373 the ¹⁴C was excreted as ¹⁴CO₂ in the expired air, while the remainder was incorporated into body
1374 proteins.

1375 When aspartame was radiolabelled at the methyl group, like ¹⁴C-methylphenylalanine, the compound
1376 was rapidly hydrolysed by esterases in the gastrointestinal tract, and the methyl group was handled by
1377 the body in a manner equivalent to the methyl group of methanol.

1378 In rabbits, unlabelled aspartame was administered daily for 4 days (20 mg/kg bw/day) and a 8.2 mg/kg
1379 bw dose of [¹⁴C(U)-phe]-aspartame was given the following day (E18, 1972). As in mice and rats,
1380 aspartame was hydrolysed in the gut before absorption of phenylalanine. A biphasic mean plasma
1381 radioactivity curve was observed, the authors suggested a rapid appearance of the phenylalanine
1382 moiety of aspartame as a plasma peptide fraction with a short half-life. Aspartame was metabolised in
1383 a fashion qualitatively similar to the other species. However, the cumulative excretion of ¹⁴CO₂ by the
1384 rabbit (7 %) was lower than in rat or monkey (17%).

1385 In a further study (E80, 1974), plasma metabolites were isolated and identified in rabbits given
1386 [¹⁴C(U)-phe]-aspartame. Since the plasma metabolic profile was similar to that identified in other
1387 species, the authors concluded that the differences observed in plasma kinetics would be due to
1388 differences in the rates of digestion and absorption in rabbits.

1389 In dogs, unlabelled aspartame (20 mg/kg bw/day) or phenylalanine (11.2 mg/kg bw/day) were
1390 administered for 5 days; on the following day, animals received 20 mg of [¹⁴C(U)-phe]-aspartame or
1391 11.2 mg of ¹⁴C(U)-phenylalanine by gavage (E17, 1972). The administered aspartame was hydrolysed
1392 in the gut (extent of hydrolysis not quantified) and the resulting radiolabelled amino acid
1393 (phenylalanine) was handled by the body identically to phenylalanine administered alone or ingested
1394 in the diet. No unchanged aspartame was detected in plasma of dogs receiving aspartame. The
1395 biological half-life of plasma ¹⁴C after oral administration of [¹⁴C(U)-phe]-aspartame was about 12
1396 days. The authors state, this value would be consistent with reported half-lives for various plasma
1397 proteins in experimental animals and in human volunteers.

1398 In rhesus monkeys, [¹⁴C(U)-phe]-aspartame, [¹⁴C(U)-CH₃]-aspartame, ¹⁴C(U)-phenylalanine or ¹⁴C-
1399 methanol were given as separate single oral doses of 0.068 mmole/kg (20 mg/kg for aspartame) (E15,
1400 1972). These data demonstrated that monkeys metabolised phenylalanine via the same metabolic route
1401 as rats. Little phenylalanine was excreted in the urine or as ¹⁴CO₂ in the expired air and the absorbed
1402 labelled material entered the normal metabolic pathways for phenylalanine. In monkeys receiving
1403 [¹⁴C(U)-CH₃]-aspartame or ¹⁴C-methanol, the kinetics of the methyl group metabolism illustrated the
1404 similarity with which monkeys and rats metabolise this moiety of aspartame. The methyl moiety was
1405 hydrolysed by esterases in the intestine, absorbed, and metabolised in the carbon pool of the body. The
1406 only difference appeared to be a slower hydrolysis in the gut of the monkey compared to the rat.

1407 In a further study, monkeys were administered unlabelled aspartame (20 mg/kg bw/day) or aspartic
1408 acid (9 mg/kg bw) orally for 5 days; on the following day, the animals received 20 mg/kg of [¹⁴C(U)-
1409 asp]-aspartame or 9 mg/kg of ¹⁴C(U)-aspartic acid by gavage (E17, 1972). Results clearly indicated
1410 that aspartate from aspartame was released by hydrolysis of the parent compound in the gut and that
1411 the free amino acid was metabolised in the same way as the dietary aspartic acid.

1412 The data of these early reports on pharmacokinetics of aspartame (E15, 1972; E17, 1972; E18, 1972;
1413 E80, 1974) showed that radioactivity associated with unchanged aspartame was not detectable in
1414 plasma of experimental animals. Based on the sensitivity of the detection of radioactivity following
1415 labelling of the phenylalanine, aspartate and methyl moieties of aspartame, it can be concluded that
1416 aspartame is completely hydrolysed in the gut to yield aspartate, phenylalanine and methanol. These
1417 metabolites are then absorbed and enter normal endogenous metabolic pathways.

1418 3.1.2. Additional studies on absorption, distribution, metabolism and excretion

1419 In addition to these basic kinetic studies, further studies (summarised below) have been performed
1420 elucidating specific aspects of absorption, distribution, metabolism and excretion.

1421 Studies in rats, dogs and monkeys have shown that after oral ingestion, aspartame is hydrolysed, either
1422 within the lumen of the gastro-intestinal (GI) tract, or within the mucosal cells lining the GI-tract.
1423 While aspartame in acidic aqueous solutions under *ex vivo* conditions has a half-life of several days
1424 (Pranker *et al.*, 1992), its half-life in the GI tract is in the order of minutes. The accelerated
1425 hydrolysis of aspartame in the GI-tract is mediated by specialised enzymes in the intestine, including
1426 esterases and peptidases (especially aminopeptidase A) (Oppermann, 1984; Hooper *et al.*, 1994). The
1427 products that result from these reactions are methanol and the amino acids, aspartic acid and
1428 phenylalanine. Hydrolysis of aspartame releases a maximum of 10% methanol by weight (Magnuson
1429 *et al.*, 2007). Hydrolysis is very efficient and the amount of aspartame that enters the bloodstream has
1430 been reported as undetectable in several studies (Oppermann, 1984; Burgert *et al.*, 1991).

1431 Burton *et al.* (1984) investigated the fate of phenylalanine methyl ester in two female rhesus monkeys
1432 fitted with hepatic portal vein cannulae. Following intragastric and intraduodenal administration of 20
1433 mg doses of [¹⁴C(U)-phe]phenylalanine methyl ester hydrochloride, small amounts of unchanged
1434 phenylalanine methyl ester were detected during the first 1-2 hour after administration, but none was
1435 detectable (<0.001 µg/ml) at later times. When comparing the areas under the phenylalanine methyl
1436 ester and total radioactivity blood concentration-time curves, the authors concluded that only 0.2% of
1437 the administered phenylalanine methyl ester reached the portal blood unchanged and 0.1% or less
1438 reached the peripheral blood unchanged. Additional *in vitro* work showed that intestinal mucosa
1439 homogenates, blood and plasma rapidly metabolise phenylalanine methyl ester to phenylalanine and
1440 methanol (Burton *et al.*, 1984).

1441 The intestinal metabolism of aspartame and L-phenylalanine methyl ester in the gut lumen was further
1442 studied in a young pig model *in vitro* and *in vivo* (Burgert *et al.*, 1991). In the *in vivo* studies, 6 young
1443 female Chester white pigs (10-15 kg) were catheterised and had a 10-French double lumen tube with a
1444 proximal port opening in the proximal jejunum and the distal port 30 cm further down the intestine. At
1445 least three days were allowed for recovery after surgery. In bolus dosing studies, compounds (L-

1446 phenylalanine methyl ester, aspartame or L-phenylalanine) were administered at a dose of 2.5
1447 mmol/kg in a Latin square design. For aspartame, this corresponded to 735 mg/kg bw. Portal vein and
1448 vena cava blood samples were taken over a period of 4 hours following dosing, and each animal
1449 received all three compounds with three days for recovery between doses. In steady state perfusion
1450 studies, equimolar solutions (25 mM) of each compound were administered directly into the jejunum
1451 at a rate of 5ml/min and the perfusate was analysed.

1452 In the bolus dosing studies, no intact L-phenylalanine methyl ester, aspartame or
1453 aspartylphenylalanine were detected in blood samples from either the portal vein or the inferior vena
1454 cava after dosing with L-phenylalanine methyl ester, aspartame or L-phenylalanine. No significant
1455 differences were observed with regard to AUC in the concentration of phenylalanine in portal blood
1456 after dosing with L-phenylalanine methyl ester, aspartame or L-phenylalanine. A similar pattern was
1457 observed in vena caval blood although the absolute phenylalanine concentration detected and
1458 corresponding AUCs were lower by 15%. Portal blood tyrosine concentration was similar after all
1459 three compounds but lower than phenylalanine concentration (mean peak values of 167 μ M for
1460 tyrosine vs 2510 μ M for phenylalanine).

1461 Neither portal nor vena caval methanol levels changed after bolus administration of phenylalanine, but
1462 values in portal blood increased from a baseline of 0 mM to 3.50 ± 0.75 mM after aspartame and 3.25
1463 ± 0.65 mM after phenylalanine methyl ester, while vena caval levels peaked at 3.26 ± 0.65 mM after
1464 aspartame and 3.13 ± 0.75 mM after phenylalanine methyl ester. Thus, the vena caval values were
1465 slightly, but not significantly, lower than those in the portal circulation.

1466 In steady state perfusion studies, direct infusion of aspartame into the jejunum resulted in the
1467 appearance of methanol, aspartic acid, phenylalanine and aspartylphenylalanine in the perfusate, with
1468 the concomitant disappearance of aspartame from the perfusate (1.46 μ mol/min/cm gut). L-
1469 Phenylalanine methyl ester was also present at detectable levels. Perfusion with L-phenylalanine
1470 methyl ester itself resulted in the appearance of phenylalanine and methanol in the perfusate. Perfusion
1471 with L-phenylalanine resulted in rapid disappearance of phenylalanine but not any of the other
1472 compounds in the perfusate. From the steady state perfusion data the authors concluded that aspartame
1473 was hydrolysed to methanol and aspartylphenylalanine in the intestinal lumen (Burgert *et al.*, 1991).

1474 In a study by Trocho *et al.* (1998), [14 C-methyl]-aspartame was administered to male Wistar rats (1-5
1475 animals per time point investigated) at a single oral dose level of 68 μ mol/kg bw (20 mg/kg bw) and
1476 122 μ Ci/kg bw by gavage. The authors reported that in liver, kidney and plasma about 0.1–0.4% of the
1477 dose of radioactivity/g tissue was detected at 1 hour and persisted between 6-24 hours. In particular, in
1478 blood (0.1-0.2% of the dose of radioactivity/g tissue) approximately 98% of the radioactivity was
1479 incorporated into protein and in the liver about 78% was incorporated to protein or nucleic acids at 6
1480 hours. In an additional study, two rats were administered radiolabelled [14 C-methyl]-aspartame (37
1481 MBq, 85.5 μ moles) orally after pre-treatment for 2 days with unlabelled aspartame. At an unspecified
1482 time point, plasma was collected and subjected to acid precipitation followed by hydrolysis (6N HCl,
1483 48 hours), liberating amino acids from proteins. The hydrolysate was then filtered to remove 'Maillard
1484 adducts'. Maillard adducts are produced through the reaction of sugar carbonyl groups with amino
1485 groups (in, for example, amino acids) and would have been produced during the hydrolysis procedure
1486 and therefore as artefacts associated with sample processing. The authors reported that the filtered
1487 Maillard adducts were radioactive. The filtered amino acids – assumed not to contain any Maillard
1488 adducts - were then derivatised with dinitrofluorobenzene and analysed by 2D thin layer
1489 chromatography.

1490 The authors present data to show that a single radiolabelled species was observed in plasma after
1491 hydrolysis and derivatisation. The authors speculated that the bound radioactivity resulted from the
1492 formation of formaldehyde from aspartame-derived 14 C-methanol (Trocho *et al.*, 1998).

1493 The study of Trocho *et al.* (1998) has been criticised. For example, Tephly (1999) argued that the
1494 adducts were not identified with certainty and adduct formation with ¹⁴C-methanol or ¹⁴C-
1495 formaldehyde were not studied *in vivo* to allow adequate comparison.

1496 The Panel agreed with this criticism and further noted that the methodology used by Trocho was not
1497 able to differentiate between ¹⁴C incorporated into proteins through the metabolic one carbon
1498 tetrahydrofolate pathway and direct covalent reaction (e.g. through Schiff base formation) of
1499 formaldehyde with proteins. The authors failed to compare the single radioactive species obtained
1500 after hydrolysis of plasma protein and derivatisation of amino acids, with several of the amino acids
1501 susceptible to reaction with formaldehyde. This did not exclude the possibility that the single
1502 radioactive species in hydrolysates was not a trace level of a Maillard adduct (and therefore an artefact
1503 from sample processing) as well as omitting other controls. The Panel therefore concluded that the
1504 work of Trocho *et al.* (1998) does not demonstrate unequivocally that formaldehyde from aspartame-
1505 derived methanol reacts with tissue macromolecules.

1506 3.1.3. Human Studies

1507 3.1.3.1. Single Dose Administration Studies

1508 The pharmacokinetics and metabolism of [¹⁴C(U)-phe]-aspartame were determined in three healthy
1509 male adults receiving a single dose of 500 mg aspartame/person in water (E15, 1972). Results
1510 demonstrated that the radiolabel from aspartame was handled in a fashion similar to that of the natural
1511 amino acid phenylalanine. As in mice, rats, rabbits, dogs and monkeys, aspartame was hydrolysed in
1512 the intestine (extent of hydrolysis not quantified), and the resulting ¹⁴C-phenylalanine was rapidly
1513 absorbed from the gut and was detected in the plasma as naturally occurring polar components
1514 comprising a mixture of proteins, peptides and free amino acids. Both qualitatively and quantitatively
1515 the metabolites of [¹⁴C(U)-phe]-aspartame found in the plasma appeared to be the same as those
1516 detected in the other animal species studied.

1517 When human volunteers were given a single oral dose of 34 mg aspartame/kg bw, no detectable
1518 increase in the blood methanol concentration was observed (limit of detection: 3.5-4 mg/L) (Tephly
1519 and McMartin, 1984). When adults were given a dose of 50 mg aspartame/kg bw, peak blood levels
1520 were around the limit of detection. With doses of 100, 150 or 200 mg aspartame/kg bw, peak blood
1521 levels of 13, 21 and 26 mg methanol/L blood were observed between 1–2 hour post dosing. Following
1522 a dose of 100 mg/kg bw, the blood methanol returned to undetectable levels at 8 hour post dosing.
1523 With the 150 and 200 mg/kg bw doses, methanol persisted longer in the blood, but at 24 hours post
1524 dosing, blood methanol was below the limit of detection. Both blood peak levels and blood AUCs
1525 were proportional to the dose. The values reported are consistent with the known kinetics of methanol
1526 (Horton *et al.*, 1992).

1527 Plasma levels of formate and the (reactive) formaldehyde intermediate metabolite have also been
1528 examined (Stegink, 1984). After giving aspartame to human volunteers at oral dose levels up to 200
1529 mg/kg bw, no clear increase in the blood concentration of formic acid could be observed because of
1530 the high background before dosing and the high variability of the value 3 hours and 24 hours after
1531 dosing. At this dose (200 mg/kg bw), the background level of blood formic acid was 18 ± 5.3 mg/L,
1532 22 ± 11 mg/L 3 hours after dosing and 11 ± 8.4 mg/L 24 hours after dosing (values derived from Fig
1533 28, Stegink, 1984). Renal excretion of formic acid was higher by 2-3-fold over basal levels (34 ± 22
1534 $\mu\text{g}/\text{mg}$ creatinine) during the 0-8 hour period following aspartame intake.

1535 The same author (Stegink, 1984) reported a series of experiments in humans to investigate the dose
1536 relationships between oral intake of aspartame and systemic plasma levels of aspartic acid,
1537 phenylalanine and methanol. The relationships were studied for doses ranging from 0-200 mg
1538 aspartame/kg bw/day, and the studies focused on blood levels that occurred following one single dose
1539 of 34 mg/kg bw/day, because at that time, this was the 99-percentile of the 'projected' daily

1540 ingestion²⁴. Plasma phenylalanine, aspartate and methanol concentrations were measured in normal
1541 subjects ingesting aspartame at 34, 50, 100, 150 and 200 mg/kg bw in a randomised crossover study.

1542 Following administration of aspartame at a dose level of 34 mg/kg bw, plasma phenylalanine levels
1543 increased significantly ($p \leq 0.001$) from a normal baseline level of 50-60 μM to $110 \pm 25 \mu\text{M}$. At a
1544 dose of 50 mg/kg bw of aspartame, plasma phenylalanine levels increased significantly ($p \leq 0.001$)
1545 from baseline values reaching a mean (\pm SD) peak value of $162 \pm 49 \mu\text{M}$. Mean (\pm SD) peak plasma
1546 phenylalanine levels were 203 ± 20.5 , 351 ± 113 and $487 \pm 151 \mu\text{M}$ following administration of
1547 aspartame at 100, 150 and 200 mg/kg bw, respectively, and were statistically higher than baseline
1548 levels. All peak values were reached between 30 min and 2 hours following dosing.

1549 Following administration of aspartame at a dose of 34 mg/kg bw and 50 mg/kg bw, no significant
1550 differences from baseline values of $2.2 \pm 0.8 \mu\text{M}$ were noted in plasma aspartate. The mean (\pm SEM)
1551 peak plasma aspartate levels (after 0.5 hour from aspartame administration) were $4.3 \pm 2.3 \mu\text{M}$
1552 ($p < 0.02$) (at 100 mg/kg bw dose), $10.0 \pm 7.0 \mu\text{M}$ (at 150 mg/kg bw dose) (statistically not significant)
1553 and $7.6 \pm 5.7 \mu\text{M}$ (at 200 mg/kg bw dose) (statistically not significant), showing that aspartate levels
1554 did not appear to be dose-dependently increased. The authors concluded that these data indicate that
1555 rapid metabolism of the aspartate portion of aspartame occurred at all doses studied (Stegink, 1984).

1556 Peak plasma levels of amino acids after aspartame intake have been compared to peak plasma levels
1557 after ingestion of dietary proteins. It was noted that amino acids from proteins in meals were liberated
1558 in the gut and absorbed more slowly than the amino acids in small peptides such as aspartame (EFSA
1559 2006).

1560 Stegink *et al.* (1979a) measured plasma, erythrocyte and milk levels of free amino acids in six normal
1561 female subjects with established lactation after oral administration of either aspartame or lactose at 50
1562 mg/kg body weight in a cross-over study. No significant change in plasma or erythrocyte aspartate
1563 levels was noted following aspartame or lactose administration. Plasma phenylalanine levels increased
1564 approximately four-fold over fasting values 45 minutes after aspartame loading ($P < 0.001$), and
1565 returned to baseline by 4 hours. Milk phenylalanine, but not aspartate, levels were increased between 1
1566 and 12 hours after aspartame intake.

1567 3.1.3.2. Studies in adults heterozygous for phenylketonuria and other sub-populations

1568 In addition to the aforementioned studies investigating the metabolism of aspartame in healthy adult
1569 volunteers, there are a number of additional absorption, distribution, metabolism and excretion
1570 (ADME) studies on aspartame that were conducted in one-year-old infants, lactating women, in
1571 individuals who respond adversely to monosodium glutamate (MSG; so-called 'Chinese Restaurant
1572 Syndrome'), in adults heterozygous for phenylketonuria (PKU) and the elderly.

1573 The study by Filer *et al.* (1983) shows that the metabolism of a single dose of aspartame (up to 100
1574 mg/kg bw, measured as peak plasma levels and AUC for phenylalanine and aspartate) given orally to
1575 one-year-old infants was not different from that measured in adults. Likewise, the metabolism of a
1576 single oral dose of aspartame of 50 mg/kg bw in lactating women (measured as plasma phenylalanine
1577 and aspartate levels) was not different from other adults (Stegink *et al.*, 1979a; E93, 1977). There was
1578 also no apparent difference in aspartame metabolism in individuals who respond adversely to
1579 monosodium glutamate (E110, 1979).

²⁴ Projected levels of aspartame ingestion have been calculated by the FDA, the Market Research Corporation of America (MRCA), and by Stegink research group. If aspartame totally replaces estimated mean daily sucrose intake, aspartame intake would range between 3 and 11 mg/kg bw with the highest daily aspartame ingestion would vary from 22 to 34 mg/kg bw. The latter is equivalent to ingesting 12-19 mg/kg bw phenylalanine, 9.8-15.2 mg/kg bw aspartate and 2.4-3.7 mg/kg bw methanol. According to the MRCA, an aspartame intake of 34 mg/kg bw represented the 99th percentile of projected daily ingestion (calculation carried out before approval of aspartame for use in carbonated beverages).

1580 Several studies on the metabolism of aspartame in adults heterozygous for PKU were conducted using
1581 single doses ranging from 10 to 100 mg aspartame/kg bw (E108, 1978; E109, 1978; Stegink *et al.*,
1582 1979b; Stegink *et al.*, 1980; Caballero *et al.*, 1986; Stegink *et al.*, 1987; Filer and Stegink, 1989,
1583 Curtius *et al.*, 1994). In two studies (Stegink *et al.*, 1979b, 1981a) fasted subjects heterozygous for
1584 PKU were compared with fasted normal subjects following a dose of 34 mg aspartame/kg bw
1585 (dissolved in orange juice). In heterozygotes, basal plasma phenylalanine levels and the phenylalanine
1586 levels following administration of aspartame were significantly higher and the plasma concentration-
1587 time curve broader than those recorded in normal subjects. While maximal plasma phenylalanine
1588 levels in healthy normal adults ($111 \pm 24.9 \mu\text{M}$) were in the range observed postprandially (Stegink,
1589 1984), maximal plasma phenylalanine levels in subjects heterozygous for PKU were $160 \pm 22.5 \mu\text{M}$,
1590 and therefore were slightly above the normal postprandial level. The plasma phenylalanine
1591 concentration-time curve was also significantly greater in heterozygous female subjects (21.36 ± 5.10
1592 IU) than in normal female subjects (10.84 ± 2.32 IU). In a further study (Stegink *et al.*, 1980) subjects
1593 known to be heterozygous for PKU and subjects assumed to be normal were administered a dose of
1594 aspartame of 100 mg/kg bw (dissolved in orange juice). Plasma phenylalanine levels in heterozygous
1595 subjects after aspartame loading were significantly higher than values in normal subjects from 30 to
1596 180 min, and the area under the plasma concentration-time curve greater. The maximal plasma
1597 phenylalanine level in heterozygous subjects after aspartame administration was $417 \pm 23.5 \mu\text{M}$ vs
1598 $202 \pm 67.7 \mu\text{M}$ in normal subjects. The authors concluded that fasted heterozygotes given an acute
1599 dose of aspartame metabolised the phenylalanine part of aspartame approximately half as fast as
1600 normal subjects. In another study (Curtius *et al.*, 1994), the effect of a protein-rich meal alone or in
1601 combination with 25 mg aspartame/kg bw on plasma phenylalanine levels in normal subjects and
1602 subjects known to be heterozygous for PKU was investigated. In meals supplemented with aspartame
1603 (25 mg/kg bw), the highest mean plasma phenylalanine levels were only slightly higher than the
1604 postprandial range for normal subjects (values for normal subjects were not reported but the Panel
1605 estimated mean values from the graph in the publication to be $80 \mu\text{M}$ vs $110 \mu\text{M}$). In the case of
1606 subjects known to be heterozygous for PKU, the increase was from a postprandial level of 126 ± 21
1607 μM to $153 \pm 21 \mu\text{M}$.

1608 A study that compared young with elderly adult volunteers revealed that following a single dose of 40
1609 mg aspartame/kg bw, C_{max} and AUC values for plasma phenylalanine were modestly but
1610 significantly higher in the elderly due to a rise in the elimination half-life of phenylalanine from 3.5 to
1611 3.9 hours (Puthrasingam *et al.*, 1996).

1612 3.1.3.3. Repeat Dose Administration Studies

1613 Several studies were conducted to address the effects of repeated ingestion of aspartame on its
1614 metabolism, and plasma phenylalanine and aspartate levels, and are summarised as follows. The
1615 ingestion by volunteers of a 600 mg aspartame dose every hour for eight hours resulted in significant
1616 increases in plasma phenylalanine and tyrosine levels, reaching a plateau after several doses (five
1617 doses for phenylalanine levels, three to six doses for tyrosine levels) (UN05, 1987; Stegink *et al.*,
1618 1989). However, the plateau levels did not exceed the normal postprandial range in plasma and
1619 returned to baseline values by 24 hours. The peak ratios of phenylalanine levels to large neutral amino
1620 acids (LNAA) levels were increased compared to the placebo in the subjects, similar to that observed
1621 following a single 34 mg/kg bw dose of aspartame. Similarly, the peak tyrosine/LNAA ratio was
1622 increased. The Panel noted that in this study, volunteers received hourly doses of 600 mg aspartame
1623 for 8 hours each day, a daily dose of 4800 mg or 80 mg/kg bw/day. This total dose corresponded to the
1624 administration of two ADIs per day. Since the plateau levels of phenylalanine did not exceed the
1625 normal postprandial range, the Panel considered that there is no accumulation of phenylalanine
1626 resulting from this high dose of aspartame.

1627 Healthy adults (108 volunteers) were given either placebo or 75 mg/kg bw/day aspartame in three
1628 divided doses for 6 months (Leon *et al.*, 1989). Throughout the treatment period, there were no
1629 changes in phenylalanine, aspartate or methanol plasma concentrations, in phenylalanine/LNAA ratio
1630 and in urinary formate excretion. The Panel noted that in this study healthy adults received almost

1631 twice the ADI for 6 months without any consequences in terms of phenylalanine or methanol
 1632 accumulation. In further studies, the effect of repeated ingestion of aspartame (in increasing doses
 1633 from 600 up to 8100 mg per person per day) over periods of up to 27 weeks on plasma amino acid
 1634 levels were also investigated in obese subjects, insulin-dependent and -independent diabetic subjects
 1635 and in children (E23, 1972; E24, 1972; E60, 1973; E61, 1972; E64, 1972). None of the studies showed
 1636 a significant difference in plasma phenylalanine or aspartate levels compared to controls.

1637 Stegink *et al.* (1990) showed that the hourly ingestion of 600 mg aspartame by adults heterozygous for
 1638 PKU over a period of 8 hours significantly increased plasma phenylalanine levels, reaching a plateau
 1639 after five doses (see also UN05, 1987). The highest mean value was $165 \pm 34.4 \mu\text{M}$ at 7.5 hours,
 1640 which remains within the normal postprandial range. Phenylalanine plasma levels returned to baseline
 1641 values by 24 hours. The peak ratio of phenylalanine levels to LNAA levels, but not the peak
 1642 tyrosine/LNAA ratio, was increased compared to the placebo. In another study (E25, 1972), aspartame
 1643 was administered in increasing doses from 600 up to 8100 mg person per day to adults heterozygous
 1644 for PKU for six weeks. No statistically significant difference in plasma phenylalanine levels were
 1645 observed between placebo controls and aspartame-exposed subjects. In a follow-up to this study,
 1646 adults heterozygous for PKU were given 1800 mg aspartame per day (600 mg doses three to times
 1647 daily) over 21 weeks. Again, there was no difference between placebo- and aspartame-exposed
 1648 individuals in terms of plasma phenylalanine levels except for a small but statistically significant
 1649 difference at week 16 (107 vs 126 μM for control and aspartame, respectively).

1650 Overall, the Panel noted that in all the repeat dose administration studies, healthy adults, children or
 1651 patients suffering various diseases including heterozygous for PKU received daily dose of aspartame
 1652 above the current ADI of 40 mg/kg bw/day for periods up to 6 months. Considering the absence of a
 1653 significant increase in plasma phenylalanine concentration from the normal postprandial range, the
 1654 Panel concluded that in line with normal uses and use levels there is no risk of phenylalanine
 1655 accumulation in such human populations.

1656 3.1.4. β -Aspartame

1657 β -Aspartame (β -L-aspartyl-L-phenylalanine methyl ester) is formed to a minor extent during the
 1658 manufacturing process and in storage of aspartame-containing beverages. In a series of studies, the
 1659 pharmacokinetics and metabolism of orally administered ^{14}C - β -aspartame were determined in humans
 1660 and in various animal species. In all these studies, ^{14}C - β -aspartame was radiolabelled on the
 1661 phenylalanine moiety.

1662 In humans, six healthy subjects received a single oral dose of ^{14}C - β -aspartame dissolved in water (31.9
 1663 or 40 mg, corresponding to 0.5 – 0.7 mg/kg bw) and were observed for 7 days (E172, 1987; E173,
 1664 1988). The use of HPLC enabled quantification of both the unchanged β -aspartame and its major
 1665 metabolites in plasma, urine and faeces. The authors reported that more than 90% of the orally
 1666 administered radioactivity was absorbed based on the low recovery of radioactivity excreted in faeces
 1667 for 7 days (9.6% of the radioactive dose). Unchanged ^{14}C - β -aspartame was not detected in plasma and
 1668 less than 0.15% of the dose was excreted unchanged in the urine after 7 days. The demethylated
 1669 metabolite (^{14}C - β -L-aspartyl-L-phenylalanine) was the major metabolite observed in plasma (T_{max} =
 1670 1.5 h, plasma half-life = 1.1 h) and was an important urinary metabolite (7% of the administered
 1671 radioactivity). In plasma, phenyl-acetyl-glutamine was detected as another major circulating
 1672 metabolite whereas free ^{14}C -phenylalanine appeared as a minor one. The major route of excretion of
 1673 radioactivity was urine (42% of the radioactive dose). The major urinary metabolite was ^{14}C -phenyl
 1674 acetyl glutamine, its excretion accounted for 30.8% of the radioactive dose. ^{14}C -phenylalanine was the
 1675 only metabolite recovered in faeces. There were no clinical effects related to ^{14}C - β -aspartame
 1676 administration at these dose levels in healthy subjects.

1677 In rabbits (E170, 1986), dogs (E169, 1987) and monkeys (E171, 1985) similar data were obtained
 1678 including the absence of unchanged ^{14}C - β -aspartame in plasma and the percentages of excretion in
 1679 urine and faeces. In these animal species, 4 to 5% of the radioactive dose was excreted in the breath.

1680 The Panel noted that these studies indicated that there is a negligible systemic bioavailability of the
1681 unchanged β -aspartame. The Panel also noted the low plasma level of free phenylalanine subsequent
1682 to the administration of β -aspartame and that the major urinary metabolites are phenyl-acetyl-
1683 glutamine and β -L-aspartyl-L-phenylalanine, a normal constituent of human urine (Burton *et al.*,
1684 1989).

1685 **3.1.5. Overall summary of the ADME data**

1686 Overall, the Panel noted that no unchanged aspartame was identified in body fluids or in tissues from
1687 experimental animals or humans in any of the studies. Therefore, the Panel concluded that after oral
1688 ingestion, aspartame was hydrolysed in the gastrointestinal tract to yield aspartic acid, phenylalanine
1689 and methanol. These metabolites are then absorbed and enter normal endogenous metabolic pathways.
1690 In humans, only subjects heterozygous for PKU showed a somewhat reduced capacity to metabolise
1691 the phenylalanine moiety of the aspartame molecule.

1692 **3.2. Toxicological data of aspartame**

1693 The content of DKP in the aspartame used in these studies, as reported by the authors of the original
1694 reports, varied from 0.1% to 4.0% per batch of aspartame; the aspartame dose was not corrected for
1695 this. In some of the unpublished reports, the DKP content is not reported.

1696 **3.2.1. Acute toxicity of aspartame**

1697 **3.2.1.1. Acute toxicity studies received following a public call for data which were available at** 1698 **the time of the previous JECFA and SCF evaluations**

1699 The acute toxicity of aspartame was studied in three species, mice, rats, and rabbits (E46, 1973).
1700 Aspartame was administered by gavage (as 15-25% suspension in 1% Tween 80) at doses up to 5000
1701 mg/kg bw (E46, 1973) or by i.p. injection (in mice and rats only). Male Schmidt Ha/ICR mice (n=6)
1702 were dosed by gavage at 1000 or 5000 mg/kg bw or by i.p. injection at 200 or 450 mg/kg bw (n=2) or
1703 1000 mg/kg bw (n=6) (E46, 1973). Male Sprague Dawley rats (n=6) were dosed by gavage at 5000
1704 mg/kg bw or by i.p. injection at 2033 mg/kg bw. Male New Zealand White Luenberg rabbits were
1705 dosed by gavage at 2000, 2500 or 3200 mg/kg bw (n=1) and 4000 or 5000 mg/kg bw (n=3) (E46,
1706 1973). The animals were observed intermittently during the 7 day post-treatment period and no
1707 remarkable motor or behavioural activities were noted. No mortalities were observed in the
1708 experimental period. According to the authors, the LD₅₀ values were in excess of the highest doses
1709 administered to each species.

1710 Male Charles River CD rats (10 weeks of age; 6 animals/group) received 100 mg/kg of aspartame by
1711 intravenous injection (5 ml/kg bw of 2% aspartame in 0.9% aqueous sodium chloride solution
1712 administered into the jugular vein over a period of 10 minutes); control animals received an equivalent
1713 volume of sodium chloride (E84, 1974). The animals were monitored for a 72-hour period post-
1714 treatment, then sacrificed and representative tissues examined microscopically. Survival was 100% in
1715 both control and treated animals. Physical, ophthalmoscopic and body weight changes were
1716 unremarkable. No compound related changes were observed at the gross or microscopic level, organ
1717 weights and organ to body weight ratios were unchanged. Moderate to severe phlebitis was observed
1718 at the site of implantation of the cannula in all rats; the authors concluded that the inflammation
1719 observed in the kidneys and endocardium could be a result of an infection.

1720 Two groups of four male Beagle dogs received either 0 or 100 mg/kg of aspartame by intravenous
1721 injection (5 ml/kg injections of 0.5% aqueous sodium chloride solutions administered over a period of
1722 10 minutes) (E85, 1974). The dogs were monitored for 72 hours post-injection before sacrifice.
1723 Physical appearance, feed consumption and body weight were recorded. All animals survived during
1724 the observation period and no remarkable changes were observed. Electrocardiograms were made at 0,
1725 0.5, 3, 6, 24, 48 and 72 hours, patterns were unremarkable pre- and post-injection in both placebo and
1726 treated dogs. No statistically significant changes were observed in the haematology, clinical chemistry
1727 and urinalysis parameters evaluated. Post-mortem gross and microscopic changes were unremarkable.

1728 The overview of these studies is presented in Annex F.

1729 3.2.2. Short-term and subchronic toxicity of aspartame

1730 3.2.2.1. Studies of short-term and subchronic toxicity received following a public call for data 1731 which were available at the time of the previous JECFA and SCF evaluations

1732 Mice of the Ha/ICR strain (8 weeks of age) were randomly assigned, five per group to study groups
1733 (E2, 1972). It was planned to administer aspartame for 4 weeks at dose levels of 0, 2000, 4000 and
1734 10000 mg/kg bw/day, but due to an unforeseen increase in feed consumption, the actual intake of the
1735 test compound exceeded this by 25-30% in all the groups. The actual intake was calculated to be 3000,
1736 5000 and 13000 mg/kg bw/day in the test groups. No statistically significant difference in body weight
1737 was observed between treated and control animals. No adverse clinical conditions were noted and
1738 100% survival was reported. Upon sacrifice, no treatment-related pathological changes were reported
1739 except that the intestinal mucosa from the high-dose treated mice was heavily coated with a clear,
1740 moderately viscous fluid. Only the control animals and those from the high-dose group were
1741 examined.

1742 In a sub-acute study, aspartame was given in the diet for 4 weeks to male and female Charles River
1743 CD rats (E3, 1972). Seven-week-old rats (5 animals per dose group) were administered aspartame
1744 incorporated in the diet on a w/w basis, resulting in an exposure of 0, 2000, 4000 or 10000 mg/kg
1745 bw/day. Actual consumption was estimated to be within 10% of the proposed dose. No consistent
1746 statistically significant effect in body weight was observed, though a significant decrease in feed
1747 consumption was reported in the high-dose females at weeks 2 and 3; however, this was not reflected
1748 in body weight gain, which increased during the treatment-period. No adverse clinical conditions were
1749 reported during the study period and 100% survival was reported in both control and treated groups.
1750 Only five control animals (3 males and 2 females) and the animals from the high-dose groups were
1751 examined histopathologically. No treatment-related changes were reported except that the intestinal
1752 mucosa from the treated rats was heavily coated with a clear, moderately viscous fluid.

1753 Groups of 10 male and 10 female sexually mature Charles River CD rats were dosed for 8 weeks with
1754 aspartame incorporated into the diet, resulting in a dose of 5 or 125 mg/kg bw/day, with further groups
1755 of 10 animals acting as controls (E20, 1969). Dosages were adjusted weekly based on weight of
1756 animals and feed consumption for each sex at the end of the preceding week. Survival was reported to
1757 be 100%. No effect on body weight and feed consumption was reported. No effect on physical
1758 appearance or behaviour was observed at either test level. At sacrifice, biochemistry and pathological
1759 examination was conducted on five animals from each dose and the control group. No treatment-
1760 related changes in haematology or urinalysis were reported, organ weights were unaltered except in
1761 the high-dose males where a significantly higher liver to body weight ratio was observed for the high-
1762 dose group males compared to controls. There were no remarkable histopathological findings; bile
1763 duct hyperplasia and pericholangitis were present in the livers of animals of all three groups, with no
1764 apparent increase in severity in the treated animals.

1765 Groups of 5 male and 5 female weanling Charles River CD rats (23 days of age at commencement of
1766 study) were randomly assigned to groups receiving either basal diet or diet containing aspartame (9%
1767 w/w, average dosage of 12.2 mg/kg day), or phenylalanine (5% w/w, average dosage 6.5 mg/kg day)
1768 for 9 weeks (E4, year not reported). Phenylalanine and aspartame were consumed at progressively
1769 decreasing mean daily dosage levels of 13 to 4 mg/kg and 24 to 7 mg/kg. Two deaths occurred during
1770 the study period, one control male and one aspartame-treated male, but no physical or behavioural
1771 abnormalities were observed in the surviving animals. No significant treatment-related changes in
1772 haematology, clinical chemistry, urinalysis were recorded and no treatment-related changes in relative
1773 organ weight or pathology were observed. Reduced body weight gain was noted for both treatments
1774 and both sexes (though less marked in females); mean relative feed consumption (g/kg bw/day) was
1775 equivalent to control values; absolute (g/rat/day) feed consumption was reduced proportionally to the
1776 depressed body weight gain.

1777 Aspartame was administered in the form of gelatine capsules to groups of male and female Beagle
1778 dogs (n=2) daily for a period of 8 weeks (E21, 1969). The dogs are described as 'young' and weighed
1779 7.2 – 13.2 kg at initiation. In order to administer an appropriate dose of aspartame, capsules were
1780 prepared specifically for the individual animals calculated upon their body weight at the end of the
1781 previous week. Animals received either 5 or 125 mg aspartame/kg bw/day, further groups of male and
1782 female dogs (n=2) served as controls and received empty capsules. The dogs were described as being
1783 generally normal in appearance and behaviour throughout the study, with a variable pattern of weight
1784 loss/gain/maintenance reported. All dogs survived the study period; animals were then sacrificed at the
1785 end of the 8-week dosing regime. Upon sacrifice, no consistent effects upon organ weights were
1786 recorded and no consistent changes in haematological and clinical chemistry parameters, gross
1787 pathology and histopathology were observed.

1788 The overview of these studies is presented in Annex G.

1789 3.2.2.2. Additional studies on short-term and subchronic toxicity

1790 In two consecutive reports, the effects of aspartame on liver (Abhilash *et al.*, 2011) and brain
1791 (Abhilash *et al.*, 2013) antioxidant systems and tissue injury were studied in male Wistar rats. The
1792 animals (6/group) were dosed daily by gavage for 6 months with 3 ml of aspartame (500 or 1000
1793 mg/kg bw/day) dissolved in water; control rats received the same volume of water. A range of enzyme
1794 activities was assayed in serum, liver homogenates and brain homogenates. Histological examination
1795 was also performed on tissue from the liver and brain (left half). The authors reported that rats that had
1796 received aspartame (1000 mg/kg bw/day) in the drinking water showed a significant serum increases
1797 in activities of alanine aminotransferase, aspartate amino-transferase, alkaline phosphatase and
1798 gammaglutamyl transferase. The concentration of reduced glutathione (GSH) and the activities of
1799 glutathione peroxidase and glutathione reductase (GR) were significantly reduced in the liver of rats
1800 that had received aspartame (1000 mg/kg bw/day). The levels of GSH were significantly decreased in
1801 the two experimental groups. Histopathological examination revealed leukocyte infiltration in the
1802 livers of aspartame-treated rats (1000 mg/kg bw/day). As regards the brain, the authors reported that
1803 the concentration of GSH and GR activity were significantly reduced in rats that had received the dose
1804 of 1000 mg/kg bw/day of aspartame, whereas only a significant reduction in GSH concentration was
1805 observed in the 500 mg/kg bw/day aspartame-treated group. Histopathological examination revealed
1806 mild vascular congestion in the 1000 mg/kg bw/day group of aspartame-treated rats.

1807 The Panel noted that only six rats were used per group and that the exposure was not long-term but
1808 only 6 months. Only two doses were tested and the dose at which some statistically significant effects
1809 were reported by the authors was 25 times greater than the ADI. In addition, the changes were mild
1810 and limited both qualitatively (many markers of antioxidant system were unchanged) and
1811 quantitatively and cannot be interpreted in the absence of historical values from the institute for these
1812 rats. The Panel also noted that the method used by the authors for the determination of GSH is not
1813 specific for GSH (since it detects a variety of reduced cellular thiols). Finally, focal inflammatory
1814 infiltration of the liver is a variable (even within the same liver) but a common occurrence in rodents,
1815 particularly in the portal tract regions of the liver. This phenomenon is often observed in liver sections
1816 from control populations of rodents. The Panel considered that the histopathological findings
1817 presented in the reports did not convincingly support the conclusion of the authors.

1818 3.2.3. Genotoxicity of aspartame

1819 3.2.3.1. Studies on genotoxicity received following a public call for data which were available at 1820 the time of the previous JECFA and SCF evaluations

1821 *In vitro studies*

1822 Aspartame was tested for mutagenicity in *Salmonella typhimurium* strains TA1535, TA1537, TA1538,
1823 TA98 and TA100 both in the absence and the presence of a rat liver metabolic activation system at
1824 dose levels from 10 up to 5000 µg/plate (E97, 1978; E101, 1978) (Annex H). The Panel considered

1825 that, for both studies, the methods implemented appeared to be sufficiently robust to support the
1826 results reported. Aspartame was not mutagenic in this test system, either in the absence or in the
1827 presence of the metabolic activation system.

1828 *In vivo studies*

1829 Mutagenicity of aspartame was studied using a host-mediated assay in mice (E81, 1974). Mice were
1830 treated by gavage with 0 (control), 1000, 2000, 4000, 8000 mg aspartame/kg bw/day (as three separate
1831 doses given at two hour intervals for five consecutive days) (E81, 1974). Thirty minutes after the final
1832 dose, the animals were inoculated with *Salmonella typhimurium*, G-46, by intraperitoneal injection.
1833 Three hours later the bacteria were recovered and the peritoneal washing was evaluated for the
1834 presence of mutants. In mice, the host-mediated assay revealed no evidence for mutagenicity of
1835 aspartame. The Panel noted some discrepancies in description of doses in different sections of the
1836 report and that the test system employed has not received further validation and is presently
1837 considered obsolete and therefore, the results of the study were not included in the assessment.

1838 Two dominant lethal tests in rats (15 males/group) were reported (E40, 1973; E41, 1973). Aspartame
1839 was dosed by gavage to 21-week-old male albino rats of the Charles River CD strain at a dose level of
1840 2000 mg/kg bw given in two equally divided doses administered on the same day. Immediately
1841 following treatment, each male was mated with two sexually mature virgin females weekly for eight
1842 consecutive weeks. On gestation day (GD) 14, the mated females were sacrificed for ovarian and
1843 uterine examinations. The Panel considered that the methods implemented were thought to be
1844 sufficiently robust to support the results reported. The following parameters were analysed: paternal
1845 growth, maternal pregnancy rate, uterine and ovary examination data and incidence of fetal deaths.
1846 None of these parameters was affected by aspartame treatment.

1847 Aspartame was administered by gavage to five groups of 10 male albino rats for five consecutive days,
1848 at dose levels of 0 (control), 500, 1000, 2000 and 4000 mg/kg bw/day (E43, 1972). Twenty-four hours
1849 after the last dose, each animal was administered colcemid to arrest mitosis, and sacrificed. Bone
1850 marrow cells were prepared and evaluated for chromosome aberrations. Aspartame did not increase
1851 the normal aberration frequencies compared to the control rats. The authors concluded that aspartame
1852 was not mutagenic. The Panel considered that the methods implemented were sufficiently robust to
1853 support the results reported, but considered the study limited since mitotic indexes were not reported
1854 (Annex H).

1855 Aspartame was reported not to induce chromosome aberrations in bone marrow or spermatogonial
1856 cells after administration by gavage to rats for five days (E12, 1970). However, the dose levels applied
1857 were reported inconsistently. On page 2 of the relevant Study Report (E12, 1970) it is stated that
1858 animals received aspartame at dose-levels of 400, 800, 1200 and 1600 mg/kg bw/day. In contrast, it is
1859 reported that aspartame was administered at dose-levels of 2000, 4000, 6000, 8000 mg/kg. The Panel
1860 considered that the reported results of this study were not supported by the outcome of the methods
1861 applied (Annex H).

1862 Aspartame was tested in a host-mediated assay with rats (E44, 1972). Aspartame was administered by
1863 gavage to five groups of 10 male albino rats for five consecutive days, at dose levels of 0 (control),
1864 500, 1000, 2000 and 4000 mg/kg bw/day, given in three equally divided doses. Following the final
1865 dose, the animals were inoculated with *Salmonella typhimurium*, G-46, by intraperitoneal injection.
1866 Three hours later the bacteria were recovered, and the peritoneal washing was analysed for the
1867 presence of mutants. No statistically significant effects on mutation frequency, as compared to the
1868 control, were noted in the treatment groups. The Panel noted that the test system employed has not
1869 received further validation and it is presently considered obsolete, and therefore the results of the study
1870 were not included in the assessment.

1871 **3.2.3.2. Additional studies on genotoxicity**

1872 *In vitro studies*

1873 Aspartame was studied in *Salmonella* mutagenicity tests in the absence and in the presence of
1874 metabolic activation (Annex H). No mutagenicity was detected in strains TA98, TA100, TA1535,
1875 TA1537 or TA97 for doses up to 10000 µg/plate (NTP, 2005). The Panel considered that the methods
1876 implemented were to be sufficiently robust to support the results reported (Annex H). However, the
1877 Panel noted a deviation from OECD 471 (i.e. tester strains TA102 or WP2uvrA bearing AT mutation
1878 were not used). The authors of the study judged the small increase in mutant colonies with 30% rat
1879 liver S9 as equivocal.

1880 Rencuzogullari *et al.* (2004) tested aspartame in strains TA98 and TA100 at doses ranging from 50 to
1881 2000 µg/plate in the presence and in the absence of metabolic activation. No mutagenicity was
1882 observed. However, the Panel considered that the methods implemented were not sufficiently robust to
1883 support the results reported, due to major deviations from OECD guideline (i.e. only two tester strains
1884 were used; the highest dose-level employed was lower than 5 mg/ml).

1885 Bandyopadhyay *et al.* (2008) reported aspartame to be negative in a test with *Salmonella typhimurium*
1886 TA97 and TA100 strains (plate incorporation, with and without metabolic activation; 10, 100, 250,
1887 500, 1000 e 10000 µg/plate). The Panel considered that the methods implemented were not
1888 sufficiently robust to support the results reported, due to major deviations from OECD guideline (i.e.
1889 only two tester strains were used; the concentration intervals were too wide; no replicate experiment
1890 were performed).

1891 Aspartame was tested for DNA damaging activity in the *in vitro* primary rat hepatocyte/DNA repair
1892 assay at concentrations of 5 and 10 mM (corresponding to 1.47 and 2.94 mg/ml, respectively) (Jeffrey
1893 and Williams, 2000). The Panel considered that the methods implemented were sufficiently robust to
1894 support the results reported. Aspartame was found to be negative in this assay.

1895 Rencuzogullari *et al.* (2004) tested aspartame *in vitro* in a sister chromatid exchange (SCE) assay, a
1896 chromosomal aberration test and a micronucleus test on human lymphocytes. Dose-related and
1897 statistically significant increases were observed for chromosomal aberration at both 24 and 48 hours
1898 and for induction of micronuclei only at the highest dose-levels employed (2000 µg/ml). Negative
1899 findings were observed for SCEs. The Panel noted that it cannot be excluded that the positive findings
1900 resulted from indirect effects (non-physiological culture conditions) since pH and osmolality were not
1901 reported. This conclusion is supported by negative findings obtained for SCEs in parallel cultures;
1902 however, these are not usually induced by indirect effects. The possible involvement of an indirect
1903 mechanism in the reported clastogenic effect is also supported by the fact that the study was performed
1904 in the absence of S9 metabolism: in these experimental conditions, no DNA damaging activity of
1905 aspartame is expected, as the molecule does not show any electrophilic centre directly reactive with
1906 DNA. The Panel considered that the methods implemented were not sufficiently robust to support the
1907 results reported.

1908 *In vivo studies*

1909 In the study by Durnev *et al.* (1995) which aimed at evaluating the genotoxicity of five sugar
1910 substitutes in mice, aspartame was investigated for induction of chromosomal aberrations in bone
1911 marrow cells of C57Bl/6 mice administered aspartame by oral gavage for five days at dose-levels of
1912 40 and 400 mg/kg bw to groups of five animals. A concurrent negative control group was also
1913 included. The animals were sacrificed 6 hours after the last administration of test compound. In the
1914 final two hours, colchicine was administered by intraperitoneal injection to accumulate cells in
1915 metaphase. A minimum of 100 metaphases per animal were scored. The results obtained indicate that
1916 aspartame did not induce any increase in the incidence of chromosomal aberrations compared with
1917 negative control values. However, the Panel noted that the study was poorly reported and that a
1918 concurrent positive control animal group to show whether the test system was functioning correctly
1919 had not been included. Furthermore, the sampling of bone marrow cells 6 hours after the last
1920 administration of test compound was not adequate for chromosomal aberration analysis and dose-

1921 levels administered appear to be very low. On these grounds, the Panel considered that the methods
1922 implemented were not sufficiently robust to support the results reported.

1923 In a study by Mukhopadhyay *et al.* (2000), male Swiss Albino mice were exposed by oral gavage to a
1924 blend of aspartame and acesulfame K (ratio 3.5:1.5) at doses up to 350 mg aspartame/kg bw. The
1925 blend of the two sweeteners showed a negative outcome for chromosomal aberrations. The Panel
1926 noted that no evaluation of cell cycle progression (e.g. mitotic index) was performed. Given the
1927 limitations of the study (blend of sweeteners and no mitotic index determination), the Panel considered
1928 it to be of limited relevance for the evaluation of the genotoxicity of aspartame.

1929 An acute bone marrow micronucleus test was conducted with aspartame administered orally to male
1930 Fisher 344 rats at three daily doses of 0 (control), 500, 1000 or 2000 mg/kg bw. No increase in the
1931 number of micronucleated polychromatic erythrocytes was observed at any of the tested dose levels
1932 (NTP, 2005). The Panel considered that the methods implemented were sufficiently robust to support
1933 the results reported.

1934 Peripheral blood micronucleus tests were conducted in male and female transgenic mice (Tg.AC
1935 hemizygous, p53 haploinsufficient or Cdkn2a deficient) after 9 months of exposure to aspartame at
1936 doses ranging from 3.1 to 50 g/kg diet. The highest dose tested was equivalent to 7660 and 8180 mg
1937 aspartame/kg bw/day in males and in females, respectively (NTP, 2005). The Panel considered that the
1938 methods implemented were thought to be sufficiently robust to support the results reported (Annex H;
1939 NTP, 2005). Negative results, indicative of an absence of clastogenic activity of aspartame, were
1940 obtained in male and female Tg.AC hemizygous and Cdkn2a deficient mice and in male p53
1941 haploinsufficient mice. In female p53 haploinsufficient mice, the results of the test were judged
1942 positive by the authors of the study, based on a trend test revealing a statistically significant 2.3 fold
1943 increased frequency of micronucleated erythrocytes seen in the 50 g/kg diet group (NTP, 2005).
1944 However, the Panel noted that the incidence of micronucleated erythrocytes in female controls was the
1945 lowest among the historical control values of the same laboratory; this rendered the outcome of the
1946 trend analysis positive. Nevertheless, the observed incidence of micronucleated erythrocytes in the
1947 highest dose group fell outside the range of the historical controls. However, the Panel also noted that
1948 the reported increase in micronucleated erythrocytes was observed in one gender only. Furthermore,
1949 the effect described was observed after 9 months of administration of a daily dose, which exceeded
1950 approximately 8-fold, the highest recommended dose level for genotoxic testing according to OECD
1951 guideline 474. The Panel concluded that the findings were equivocal in the p53 transgenic strain
1952 (positive in female but not in male p53 haploinsufficient mice) but negative in the other two strains,
1953 and, overall, did not indicate a genotoxic potential for aspartame.

1954 Two studies addressing DNA damage as detectable by Comet assay are available, both following
1955 administration of aspartame by gavage.

1956 Sasaki *et al.* (2002) administered a single dose of aspartame (2000 mg aspartame/kg bw) to mice (four
1957 male/group) and analysed the stomach, colon, liver, kidney, bladder, lung, brain, bone marrow.
1958 Aspartame did not induce any significant increases in DNA migration. Based on these results, the
1959 Panel considered that aspartame was not genotoxic in the organs assayed. However, the Panel noted
1960 that for the 24 hour sampling time, a reduction in DNA migration (a marker for DNA cross-linking
1961 agents) was observed in all organs analysed, but the reduction was not significant. The Panel
1962 considered that the methods implemented were sufficiently robust to support the results reported.

1963 Bandyopadhyay *et al.* (2008) administered aspartame as a single dose of 0 (control), 7, 14, 28 and 35
1964 mg aspartame/kg bw to mice (four males/group) by oral gavage, and at the highest dose-level,
1965 aspartame was reported to induce DNA damage in bone marrow cells. However, the Panel evaluated
1966 the study as poorly reported and noted that the dose levels used were low compared to other studies
1967 reporting negative results, and that an insufficient number of cells was scored (total of 50
1968 cells/animal). Therefore, the Panel considered that the methods implemented were not sufficiently
1969 robust to support the results reported, and that no conclusion could be drawn from it.

1970 Kamath *et al.* (2010) used three endpoints to assess the genotoxic potential of aspartame following
1971 administration of 0 (control), 250, 455, 500 and 1000 mg aspartame/kg bw by gavage to mice. The
1972 authors concluded that aspartame induced (a) micronuclei in bone marrow erythrocytes, (b)
1973 micronuclei in peripheral blood and (c) chromosome aberrations in bone marrow erythrocytes.
1974 However, the number of animals and the ratio of polychromatic erythrocytes to normochromatic
1975 erythrocytes (PCE/NCE) ratio (the authors only stated that it was changed) are not given. The Panel
1976 considered that the methods implemented were thought not to be sufficiently robust to support the
1977 results reported, and that no conclusion could be drawn from it.

1978 AlSuhaibani (2010) tested aspartame for its ability to induce chromosome aberrations (CA), SCE and
1979 to affect the mitotic index (MI) in bone marrow cells of mice (five males/group; by gavage) at dose
1980 levels of 0 (control), 3.5, 35 and 350 mg aspartame/kg bw. The authors concluded that aspartame
1981 induced CA at 35 and 350 mg/kg bw, but neither dose level induced SCE nor decreased the MI. The
1982 Panel noted that an insufficient number of cells were scored (total of 50 metaphase cells/animal for
1983 CA; 30 metaphases for SCE). Furthermore, no positive control was included in the study and any
1984 supplementary information on cytotoxicity relevant for CA and SCE-analysis in the present
1985 experiment was lacking. The Panel considered that the methods implemented were not sufficiently
1986 robust to support the results reported, and that no conclusion could be drawn from the study.

1987 Karikas *et al.* (1998) reported a non-covalent interaction of excess aspartame, aspartic acid and
1988 phenylalanine with calf thymus DNA, inferred from the altered chromatographic profile of DNA. This
1989 effect was attributed to the electrostatic interaction of amino groups and the negatively charged
1990 phosphate in naked DNA. The Panel considered these findings, obtained in an acellular system in
1991 presence of excess aspartame, of minimal relevance for the evaluation of the genotoxic potential of
1992 aspartame.

1993 Meier *et al.* (1990) investigated the kinetic of formation, stability and reactivity of nitrosation products
1994 of aspartic acid, aspartame, and glycine ethyl ester. Nitrosation products were obtained *in vitro*, with
1995 incubation of 40 mM substrate and nitrite at pH 2.5 for varying times. The nitrosation products
1996 displayed an 'alkylating' activity *in vitro*, as indicated by the reactivity with the nucleophilic
1997 scavenger 4-(4-nitrobenzyl)pyridine, measured with a colorimetric method. In the same study, co-
1998 administration of glycine ethyl ester and nitrite to rats did not result in the formation of detectable
1999 DNA adducts in rat stomach.

2000 The issue of nitrosation was also addressed by Shephard *et al.* (1993). Aspartame and several naturally
2001 occurring dipeptides were nitrosated *in vitro* at low pH (3.5) in the presence of 40 mM nitrite and
2002 tested for mutagenicity in *Salmonella typhimurium* TA100. The nitrosation products of some
2003 dipeptides (Trp-Trp, Trp-Gly) and aspartame exhibited a direct mutagenic activity, which was related
2004 by the study authors to the nitrosation of their primary amino groups.

2005 Concerning the studies of Meier *et al.* (1990) and Shephard *et al.* (1993), the Panel noted the harsh
2006 conditions utilised for the *in vitro* nitrosation of substrates and considered the results of doubtful
2007 relevance for the assessment of the genotoxic risk posed by the dietary intake of aspartame or other
2008 natural amino acids and dipeptides.

2009 3.2.3.3. Conclusion on the genotoxicity of aspartame

2010 The Panel concluded that the *in vitro* genotoxicity data on bacterial reverse mutation exhibited some
2011 limitations (e.g. absence of TA102 and WP2 uvrA *Escherichia coli*). However, the Panel considered
2012 the weight-of-evidence was sufficient to conclude that aspartame was not mutagenic in bacterial
2013 systems. Concerning mammalian systems *in vitro*, the Panel concluded that, apart from the valid UDS
2014 study that was negative, no conclusion could be drawn at the gene and chromosomal level because no
2015 studies dealing with these endpoints were available.

2016 *In vivo*, the majority of investigations on systemic genotoxicity reported negative findings. Equivocal
2017 positive findings were only described in a NTP study, positive in female but not in male p53
2018 haploinsufficient mice; in two other transgenic mouse strains the results were negative.

2019 Concerning the possible site of first contact effects *in vivo*, limited data are available. However, the
2020 available *in vitro* data do not indicate a direct genotoxic activity of aspartame that might predispose to
2021 a site of first contact effect *in vivo*.

2022 Overall, the Panel concluded that the available data do not indicate a genotoxic concern for aspartame.

2023 Summary tables on the genotoxicity of aspartame are presented in Annex H.

2024 **3.2.4. Chronic toxicity and carcinogenicity of aspartame**

2025 **3.2.4.1. Studies on chronic toxicity and carcinogenicity received following a public call for data** 2026 **which were available at the time of the previous JECFA and SCF evaluations**

2027 In these chronic toxicity and carcinogenicity studies, aspartame was administered in the diet. Although
2028 the purity of aspartame used in these studies was considered to be 100%, it was reported in the studies
2029 that the content of DKP varied from 0.2% to 1.5% per batch.

2030 *Mice*

2031 Aspartame (DKP content varied from 0.8% to 1.2% per batch) was administered in the diet to groups
2032 of 36 male and 36 female ICR Swiss mice at dose levels amounting to 0 (control), 1000, 2000 and
2033 4000 mg/kg bw/day for 104 weeks (E75, 1974). Criteria evaluated were physical appearance,
2034 behaviour, body weight gain, feed consumption, survival, clinical chemistry, eye examination, organ
2035 weights, tumour incidence and gross and histopathology. There was no effect on the physical
2036 appearance and behaviour of the animals. Body weight gain for the male mice at all dose levels was
2037 significantly lower than that for the male controls but this was accompanied by reduced total feed
2038 consumption during the first year for the males at the low- and high-dose levels. At week 52
2039 differences between control and treated male group body weight values did not exceed 3% and these
2040 differences were not considered biologically meaningful. Mean terminal body weights were
2041 unremarkable for each of the male and female treatment groups. Survival in all treated groups was
2042 comparable to that of the controls. Haematological and blood chemistry analyses revealed no
2043 compound-related alterations at any exposure level compared to the control. Random fluctuations
2044 reaching statistical significance were occasionally observed but the values remained within the range
2045 of historical control data. Gross observations at necropsy did not reveal compound-related changes in
2046 any organs or tissues. Relative organ weights were unaffected except for the relative heart weight in
2047 females that was increased at the high-dose level and the relative thyroid weight that was increased in
2048 females at the low-dose level only. In males, the relative thyroid weight was increased at the low-dose
2049 level only and the relative prostate weight was decreased at the low- and mid- dose but not at the high-
2050 dose. Histopathological examination was performed on all gross lesions from all animals at each
2051 treatment level, and on 20-27 grossly unremarkable organs from control and high-dose level animals,
2052 as well as from roughly two-thirds and one-third of the animals in the mid- and low-dose level.
2053 Detailed histopathological evaluations were performed on brain and urinary bladder from all mice in
2054 the control and the three treatment groups. In no instance in either sex at any of the treatment levels
2055 was the tumour incidence for any of the types of tumours reported significantly higher than the
2056 incidence observed in the controls. Histopathological examination revealed no evidence for any
2057 treatment-related non-neoplastic changes in any organ or tissue. The authors concluded that aspartame,
2058 administered to the mouse for 104 weeks in the diet at dose levels of 1000, 2000 and 4000 mg/kg
2059 bw/day exhibited no adverse effects regarding survival rate, and that there was no evidence of an
2060 effect with respect to the incidence of neoplasms or with regard to non-neoplastic changes in any
2061 organ or tissue. The Panel agreed with this evaluation and identified a NOAEL for this study of 4000
2062 mg/kg bw/day, the highest dose level tested.

2063 *Rats*

2064 Aspartame (DKP content varied from 0% to 1.5% per batch) was administered in the diet to groups of
2065 40 male and 40 female Charles River Albino rats at dose levels of 0 (control), 1000, 2000, 4000, and
2066 8000 mg/kg bw/day for 104 weeks (E33-34, 1973). There was no evidence that the administration of
2067 aspartame resulted in any effect on the physical appearance and behaviour of the animals. Growth
2068 rates at 8000 mg/kg bw/day were significantly lower than that of controls, but this could be related to
2069 the significantly lower values for feed consumption for the first 52 weeks for the male and female rats
2070 in the 8000 mg/kg bw/day group and for the females in the 4000 mg/kg bw/day group. Survival after
2071 104 weeks was comparable to controls for all treated groups except for the females of the 8000 mg/kg
2072 bw/day group that showed statistically significant lower survival rates. Evaluation of the clinical
2073 chemistry data revealed no consistent statistically significant alterations outside the normal range of
2074 values. The results from ophthalmoscopic examinations revealed no indication of a compound-related
2075 effect. Changes in relative organ weights were not accompanied by gross nor histopathological
2076 changes and were therefore considered by the authors to be without biological significance.
2077 Histopathological examination was performed on all gross lesions from all animals at all treatment
2078 levels and on 20-25 grossly unremarkable organs from the groups at 0, 4000 and 8000 mg/kg bw/day
2079 and from roughly one fourth of the animals at the 1000 and 2000 mg/kg bw/day groups. Urinary
2080 bladder received more extensive examination since four intermittent transverse bladder sections were
2081 examined microscopically from each rat in the study. Tumour incidence for the types of tumours
2082 analysed was not statistically significantly higher in any of the dose groups of either sex than in the
2083 controls. Histopathological examination revealed no treatment related changes in brain, pituitary,
2084 spinal cord, peripheral nerve, thyroid, adrenal, salivary gland, skin, skeletal muscle, heart, liver,
2085 mesenteric lymph node, bone marrow, stomach, small intestine, large intestine, uterus, vagina,
2086 mammary gland, testis and urinary bladder. Pulmonary pneumocyte hyperplasia was slightly increased
2087 in the 8000 mg/kg bw/day group females. The ovary was unremarkable except for an increased
2088 incidence of cystic follicles at all treatment levels, probably related to an unusually low incidence in
2089 the controls. Seminal vesicle atrophy of slight to moderate degree was inconsistently present, with a
2090 slightly increased incidence observed in the 4000 and 8000 mg/kg bw/day groups (2/23, 4/23 and 6/21
2091 in control, 4000 and 8000 mg/kg bw/day groups, respectively). Acute and chronic prostatitis was also
2092 observed somewhat more frequently in treated animals but no dose-relationship was observed. Focal
2093 pancreatic fibrosis and mild atrophy were inconsistently present at an increased incidence in treated
2094 groups affecting primarily the animals in the 8000 mg/kg bw/day group. Nodular hyperplasia of the
2095 pancreas was also observed more often (5%) at this high-dose level. The authors indicated however,
2096 that all these changes were inconsistently present and lacked clear dose-response relationships. A renal
2097 pigment deposit (identified as iron-containing haemosiderin) was present in tubular and pelvic
2098 epithelial cells with increased incidence in male rats at 8000 mg/kg bw/day. Females and low-dose
2099 males were not affected. Focal hyperplasia of the renal pelvic epithelium was also present with
2100 increased incidence in the 8000 mg/kg bw/day group in males. Tubular degeneration was increased in
2101 the 8000 mg/kg bw/day group in males. The authors concluded that administration of aspartame to rats
2102 for two years did not result in adverse effects or an increased incidence of neoplasms, and produced no
2103 evidence of treatment-related non-neoplastic lesions in any organ examined except for the renal
2104 changes in males at 8000 mg/kg bw/day. Based on these observations the Panel identified a NOAEL
2105 of 4000 mg aspartame/kg bw/day in this study.

2106 In a chronic toxicity study in the rat, aspartame (DKP content varied from 0.8% to 1.2% per batch)
2107 was administered in the diet to groups of male and female Charles River albino rats at dose levels of 0
2108 (control; 60 males/60 females), 2000 (40 males/40 females) and 4000 (40 males/40 females) mg/kg
2109 bw/day for 104 weeks post-weaning (E70, 1974). These treated rats were obtained as F1 weanlings
2110 from parental animals which had been pre-treated with aspartame at the same levels for 60 days prior
2111 to mating and continued to receive the test material during mating and throughout the gestation and
2112 nursing periods. Endpoints evaluated were physical appearance, behaviour, eye examination, growth,
2113 feed consumption, survival, clinical chemistry, organ weights, tumour incidence and gross and
2114 microscopic pathology. There was no effect on the physical appearance and behaviour of the animals.
2115 Growth rates for the exposed animals were comparable to growth rates of the controls except for the

2116 high-dose in males for which growth rates were significantly lower than for controls, this was
2117 accompanied by a significantly lower feed consumption. Survival at 104 weeks was comparable to
2118 controls for both treatment groups. Clinical chemistry data revealed no consistent statistically
2119 significant alterations outside the normal range. The results from ophthalmoscopic examinations
2120 revealed no indication of a compound-related effect. Gross observations at necropsy revealed no
2121 compound-related changes in any organs or tissues. Some statistically significant changes in relative
2122 organ weights were reported but these were not accompanied by histopathological alterations and
2123 these were therefore considered by the authors to be of no biological significance. Histopathological
2124 examination was performed on all gross lesions from all animals at all treatment levels and on 20-25
2125 grossly unremarkable organs (see below) from all control and treated animals. Microscopic evaluation
2126 of eight coronal sections representing all major neuroanatomic areas of the brain was performed for
2127 each control and treated rat. Urinary bladder also received more extended examination since four
2128 intermittent transverse bladder sections were examined microscopically from each rat in the study.
2129 Incidences for the types of tumours analysed was not statistically significantly higher in any dose
2130 group compared to the control group. Histopathology revealed that at all levels administered, there
2131 were no effects on brain, spinal cord, eye, salivary gland, thyroid, lung, small intestine, large intestine,
2132 lymph nodes, peripheral nerve, skin, testis, prostate, seminal vesicle, vagina, bone, bone marrow, and
2133 mammary gland. For the stomach, the incidence of focal gastritis, mucosal ulceration, and
2134 pigmentation was slightly increased in the treated females, but these effects were considered by the
2135 authors to be of little biological relevance. Pancreatic focal acinar hypertrophy occurred more
2136 frequently among low-dose male rats than among control animals but was considered by the authors to
2137 be spontaneous in nature. Likewise, an increased incidence of ovary atrophy among the high-dose
2138 animals and a higher incidence of endometritis in low-dose survivors and high-dose non-survivors
2139 were considered by the authors to be unrelated to treatment. Pituitary chromophobe hyperplasia and
2140 cysts involving the pars distalis were observed in higher numbers of treated animals, but because of
2141 the overall low incidence of these findings, the authors did not consider them compound-related. The
2142 incidence of nodular hyperplasia of the adrenal cortex was somewhat higher for the treated males and
2143 angiectasis of the adrenal was more frequently noted for the treated females than for the respective
2144 controls. However, the authors indicated that these findings were not common to both sexes and the
2145 differences were not considered significant. The incidence of renal cortical pigmentation among
2146 treated male and female survivors and focal mineralisation among only the treated females was
2147 slightly increased as compared to the controls but the authors indicated that there was no meaningful
2148 difference between findings for the control and treated groups. The incidence of parenchymal
2149 hyperplastic nodules was higher in the livers of treated females (3/23 in the 2000 mg/kg bw/day and
2150 1/20 in the 4000 mg/kg bw/day group) when compared with controls (0/28), whereas the incidence
2151 was similar between control (2/35) and treated males (1/20 in the 2000 mg/kg bw/day and 1/18 in the
2152 4000 mg/kg bw/day group). This finding among the treated females was not considered by the authors
2153 as compound-related since the incidence for the control females was low compared to historical
2154 control data of this strain and since there was no evidence of a dose-response relationship. The only
2155 other notable finding for this organ was a slightly increased incidence of pericholangitis among the
2156 high-dose male non-survivors. The authors concluded that rats exposed to 2000 or 4000 mg
2157 aspartame/kg bw/day throughout the full pre-natal period by placental transfer, during weaning and
2158 post-weaning for 104 weeks by direct dietary administration, exhibited no adverse effects regarding
2159 survival rates or incidence of neoplasms, nor did they exhibit evidence of treatment-related non-
2160 neoplastic changes in any organ or tissue of either sex. The Panel agreed with this evaluation and
2161 identified a NOAEL for this study of 4000 mg/kg bw/day, the highest dose level tested.

2162 In an additional study (E87, 1973), a supplemental histopathological examination of all brains from
2163 these two chronic rat carcinogenicity studies (E33-34, 1973 and E70, 1974) was performed (since the
2164 initial post-mortem assessment only involved two coronal sections of brains from each rat in the high-
2165 dose and concurrent control groups with additional examination restricted to rats that exhibited gross
2166 evidence of brain lesion(s)). In the E33-34 studies, 440 animals were examined from the 104-week
2167 study (120 control and 320 treated animals), 12 neoplasms involving the brain were discovered in rats
2168 from treated groups. Their type and distribution are presented in Table 19.

2169 **Table 19:** Intracranial neoplasms as reported in rats in the 104 week oral toxicity study on aspartame
 2170 (E33-34; E87)

Intracranial neoplasms	Male (Dose mg aspartame/kg bw/day)					Female (Dose mg aspartame/kg bw/day)				
	Cont	1000	2000	4000	8000	Cont	1000	2000	4000	8000
Astrocytoma	1/60	1/40	0/40	0/40	0/40	0/60	1/40	0/40	0/40	0/40
Astrocytoma + Ependymal components	0/60	1/40	1/40	1/40	0/40	0/60	1/40	0/40	0/40	0/40
Oligodendroglioma	0/60	0/40	0/40	1/40	0/40	0/60	0/40	0/40	0/40	0/40
Ependymoma	0/60	0/40	0/40	2/40	0/40	0/60	0/40	0/40	1/40	0/40
Meningeal Sarcoma	0/60	0/40	0/40	0/40	0/40	0/60	0/40	0/40	0/40	1/40
Glioma unclassified	0/60	0/40	0/40	0/40	0/40	0/60	0/40	0/40	0/40	1/40

2171
 2172 * In the authentication review of selected materials submitted to the Food and Drug Administration
 2173 relative to application of Searle Laboratories to market aspartame (E102a, b, c) the number of animals
 2174 with neoplasm (intracranial tumour) in the control group was reported as 1 instead of 0.

2175 In the second rat study (E70, 1974), the tumourigenic potential of aspartame was assessed after
 2176 transplacental exposure, throughout gestation via maternal milk during lactation and post weaning for
 2177 104 weeks through addition to diet. The parents of the rats in this study were also pre-treated with
 2178 aspartame for 60 days prior to mating. Nine neoplasms involving the brain were identified in the 280
 2179 animals in this study. Their type and distribution are presented in Table 20.

2180 **Table 20:** Intracranial neoplasms in rats exposed to aspartame in a lifetime toxicity study (E70,
 2181 1974; E87, 1973)

Histopathological lesions	Male (Dose mg aspartame/kg bw/day)			Female (Dose mg aspartame/kg bw/day)		
	Control	2000	4000	Control	2000	4000
Astrocytoma	3/60	1/40	1/40	1/60	1/40	0/40
Ependymoma	0/60	1/40	0/40	0/60	0/40	0/40
Meningeoma	0/60	0/40	0/40	0/60	0/40	1/40

2182
 2183 These results indicated a random occurrence of intracranial neoplasms, unrelated to treatment.

2184 Based on the results of these two studies, the Panel concluded that evidence of an intracranial
 2185 tumourigenic effect was not demonstrated. The Panel noted that in treated animals of the first 104-
 2186 week study, more brain tumours originating from different tissues (astrocytoma, oligodendroglioma,
 2187 ependymoma and meningeoma) were observed. However, there was no dose relationship and males of
 2188 the high-dose group did not show any brain tumour. Moreover, with the exception of males in the
 2189 4000 mg/kg bw/day group, only one or two animals per group exhibited a brain tumour and the overall
 2190 incidence of intracranial neoplasms in the 104-week toxicity study (2.7%) was lower than the
 2191 incidence in the control group of the lifetime carcinogenicity study (3.3%). The incidence of
 2192 spontaneous brain tumours in Sprague-Dawley derived rats has been reported by Ward and Rice
 2193 (1982) to vary from 0% up to 3.3%. A similar incidence was reported by Weisburger *et al.* (1981).

2194 JECFA also evaluated these data and concluded that neither the overall incidence nor the appearance
2195 of any particular neoplasm was associated with aspartame exposure. Therefore, JECFA concluded that
2196 aspartame did not cause brain tumours in rats (JECFA, 1980). The Panel agreed with this conclusion.

2197 An additional 2-year chronic toxicity and carcinogenicity study was conducted in Wistar rats (Ishii *et*
2198 *al.*, 1981; Ishii, 1981). Wistar rats (86 males and 86 females) were administered aspartame (DKP
2199 content up to 1%) at dose levels of 0 (control), 1000, 2000 and 4000 mg/kg bw/day in the diet for up
2200 to 104 weeks; an additional group was fed 4000 mg/kg bw/day aspartame + DKP (3:1) for up to 104
2201 weeks. There was a dose-dependent depression of body weight gain at 2000 and 4000 mg/kg bw/day
2202 and at 4000 mg/kg aspartame + DKP (3:1) in males, and at all dose levels in females. This effect was
2203 correlated with decreased feed consumption. Survival was not consistently affected. In males, but not
2204 females, there was a trend for decreasing body weight in all groups including controls after one year.
2205 There were no effects on liver weight throughout the study. Clinical biochemistry and organ pathology
2206 were unremarkable and any significant changes observed were not treatment related. Subsequent
2207 detailed analysis for brain tumours showed no evidence for increased incidence of brain tumours
2208 (Ishii, 1981). One atypical astrocytoma was found in a female control rat, and two astrocytomas, two
2209 oligodendrogliomas and one ependymoma were found in the exposed groups, with no statistical
2210 difference in incidence between the groups. There was no evidence of a treatment-related effect on
2211 incidence of non-neoplastic changes other than a dose-related increase in focal mineralisation of the
2212 renal pelvis in both males and females (incidences in males: control, 1/57; 1000 mg aspartame/kg
2213 bw/day, 5/55; 2000 mg aspartame/kg bw/day, 10/60; 4000 mg aspartame/kg bw/day, 15/59; incidences
2214 in females: control, 16/59; 1000 mg aspartame/kg bw/day, 23/59; 2000 mg aspartame/kg bw/day,
2215 30/59; 4000 mg aspartame/kg bw/day, 46/60) associated with a dose-related increase in urinary
2216 calcium (Ishii *et al.*, 1981; JECFA, 1981; EFSA, 2006). The authors attributed this lesion to irritation
2217 caused by the mineral deposition and considered to be of minimal toxicological significance. The
2218 Panel noted that mineralisation in the kidney is a common finding in rats, the aetiology being
2219 associated with mineral imbalance (Lord and Newberne, 1990). Thus, there was no evidence for
2220 toxicity of aspartame and with its degradation product DKP over 2 years in rats. The Panel identified
2221 from this study a NOAEL of 4000 mg aspartame/kg bw/day. In addition, the Panel noted that the study
2222 provided information on the lack of toxicity of aspartame when administered in conjunction with
2223 DKP.

2224 A re-evaluation of all neoplastic and non-neoplastic lesions of the Ishii *et al.* (1981) study was
2225 reported (Iwata, 2006). No significant differences between controls and treated animals were identified
2226 and it was concluded that all the tumours observed could have occurred spontaneously. Increased
2227 mineralisation of the renal pelvis (as reported in Ishii *et al.*, 1981) was observed in males at 2000 and
2228 4000 mg/kg bw/day and in females at 4000 mg/kg bw/day. The original study report (Ishii *et al.*, 1981)
2229 stated that this was not related to pelvic epithelial hyperplasia or pelvic inflammatory reactions,
2230 although the follow up study reported an increase in transitional cell hyperplasia in male at 4000
2231 mg/kg bw/day. This was attributed to irritation caused by the mineral deposition and was considered
2232 by the authors of the re-evaluation to be of negligible, if any, toxicological significance. The Panel
2233 agreed with this conclusion.

2234 *Hamsters*

2235 The JECFA evaluation (JECFA 1980) also described a chronic toxicity study in hamsters. In this study
2236 groups consisting of five male and five female hamsters were fed aspartame (containing less than 1%
2237 DKP) at dietary levels of 1000, 2000, 4000 and 12000 mg/kg bw/day for 46 weeks (E27, 1972; E35-
2238 36; 1972).

2239 The Panel noted the inadequacies of this study due to short duration of the study, the limited number
2240 of animals per group and the presence of infection of the animals. The Panel agreed that this study
2241 cannot be used for the present evaluation.

2242 *Dogs*

2243 5 Month old Beagle dogs (5 animals/sex/group) were exposed to aspartame (a content of DKP up to
2244 1%) via dietary administration for 106 weeks at dose levels that amounted to 0 (control), 1000, 2000
2245 and 4000 mg/kg bw/day (E28, 1972). Physical examinations were performed at 4-week intervals and
2246 ophthalmoscopic evaluations at 24, 52, 78 and 104 weeks of treatment. Haematology, clinical
2247 chemistry and urinalysis were performed periodically for all dogs. After necropsy, representative
2248 tissues from control and all treated animals were processed for microscopic examination. Survival
2249 rates were 100% in all groups. No compound-related variations in feed consumption and body weight
2250 gain were observed. Physical examination findings were unremarkable. No changes in motor
2251 behaviour activity or in general physical appearance were noted. Periodic ophthalmoscopic
2252 examination did not reveal any treatment related changes. Haematology and clinical chemistry
2253 findings revealed no biologically meaningful treatment related alterations, although transient sporadic
2254 significant differences in several of the parameters were reported. However most values remained
2255 within physiological limits and no dose-related pattern was evident. A significant increase in excretion
2256 of urinary phenylketones was observed in some high-dose dogs at week 2, 4 and 26 of treatment, but
2257 not at all other time intervals. There was no increase in plasma L-phenylalanine levels during the 106-
2258 week treatment period. Gross and microscopic findings were unremarkable. Organ weight data were
2259 unremarkable. An extended histopathological examination of the brain tissue did not reveal any
2260 lesions (E86, 1973). The authors concluded that continuous dietary administration of aspartame at
2261 daily intake levels of 1000, 2000 and 4000 mg/kg bw/day in Beagle dogs of both sexes for 106 weeks
2262 causes no biologically meaningful alterations in body weight, feed consumption, physical
2263 examination, clinical chemistry examinations or gross and microscopic findings. The Panel agreed
2264 with this evaluation but noted the limited numbers of animals and the short duration of the study. The
2265 Panel agreed with the NOAEL of 4000 mg/kg bw/day previously proposed by JECFA.

2266 *Monkeys*

2267 The only study addressing the chronic effects of aspartame on primates is a 52 week oral toxicity
2268 study in neonatal rhesus monkeys (*Macaca mulatta*) (E32, 1972). Starting in infancy, rhesus monkeys
2269 were dosed with aspartame (containing 0.2-1.0% DKP), which was mixed with Similac milk formula
2270 and administered four times daily. Following gradual increases in the doses administered, mean daily
2271 doses of aspartame of 970 mg/kg bw/day (planned dose 1000 mg/kg bw/day; 1 male/1 female), 3010
2272 mg/kg bw/day (planned dose 3000 mg/kg bw/day; 2 males/1 female) and 3620 mg/kg bw/day (planned
2273 dose 4000-6000 mg/kg bw/day; 2 males) were attained. The actual duration of dosing ranged from
2274 210-363 days.

2275 Survival was 100% in all except the high-dose group; one monkey in this group died after 300 days
2276 treatment; the cause of death was unknown. Feed intake and growth rate were mildly reduced by
2277 treatment with aspartame. One low-dose monkey, which had an apparent congenital abnormality, was
2278 kept in the study but had reduced weight gain compared with other monkeys in the study.
2279 Haematology and clinical chemistry parameters were generally unremarkable in treated animals
2280 except for increased plasma phenylalanine and tyrosine levels, associated with increased urinary
2281 phenylketone levels, in medium and high- dose groups after six months.

2282 The main adverse effect observed in this study was that animals in both the mid- and high-dose groups
2283 started to experience grand mal seizures, the first such event occurring on day 218. Occurrence of
2284 seizures was associated with high plasma phenylalanine levels (Median dose: mean peak of 420
2285 $\mu\text{mol}/100\text{ml}$ at 28 weeks; high-dose: mean peak of 480 $\mu\text{mol}/100\text{ ml}$ at 36 weeks); similar convulsions
2286 occurred in monkeys fed L-phenylalanine in equimolar quantities. In the low-dose group, no increase
2287 in plasma phenylalanine levels was observed and no seizures occurred. Intermittent seizures occurred
2288 thereafter for the remainder of the study, usually occurring during handling. Treatment was withdrawn
2289 for the last three months of the study; no convulsions were observed during this period.

2290 All the mid-dose and high-dose monkeys in this study developed *Shigella* infections during this study,
2291 and the data reassurance programme conducted by Searle rejected the study on this basis (Rust, 1976).

2292 It should also be noted that the study included no control group; historical control data were relied
2293 upon for comparison.

2294 In summary, treatment of rhesus monkeys with high doses of aspartame for the first year of life did not
2295 have any significant effect on biochemical parameters, but animals treated with aspartame at doses
2296 above 3000 mg/kg bw/day experienced grand mal seizures beginning after about 30 weeks' treatment.
2297 The significance of this finding is not clear because all the monkeys manifesting grand mal seizures
2298 were affected by a *Shigella* infection during the study. The Panel concluded that the study provided
2299 insufficient information to conclude on chronic effects of aspartame in monkeys.

2300 **3.2.4.2. Additional long term carcinogenicity studies**

2301 Since the last evaluation of aspartame by the SCF, two new long-term carcinogenicity studies on
2302 aspartame in rats (Soffritti *et al.*, 2006, 2007; Chiozzotto *et al.*, 2011) and one in mice (Soffritti *et al.*,
2303 2010) have been reported. All three studies were performed by the European Ramazzini Foundation
2304 (ERF).

2305 In the Soffritti *et al.* (2006) study, Sprague Dawley rats (100-150 animals/sex/group) from the in-bred
2306 colony of the ERF were exposed to 0, 80, 400, 2000, 10 000, 50 000 or 100 000 mg aspartame/kg in
2307 the diet, equivalent to 0, 4, 20, 100, 500, 2500 or 5000 mg aspartame/kg bw/day, from eight weeks of
2308 age until natural death. A range of inflammatory changes was observed in a variety of organs and
2309 tissues particularly in the lungs and kidneys, in both males and females. Furthermore, an increased
2310 incidence of lymphomas/leukaemias in female rats, with a positive significant trend in both male and
2311 female rats was reported by the authors. Additional effects included an increased incidence of
2312 transitional cell carcinomas of the renal pelvis and ureter and their precursors (dysplasias), with a
2313 positive significant trend in female rats, an increased incidence of malignant Schwannomas of
2314 peripheral nerves, with a positive significant trend in male rats and an increased incidence of
2315 malignant tumour-bearing animals, with a positive significant trend in both sexes.

2316 In 2007, Soffritti *et al.* (2007) published a follow-up study in which groups of 70–95 male and female
2317 Sprague-Dawley rats were exposed to 0, 400 or 2000 mg aspartame/kg in the diet, equivalent to 0, 20
2318 or 100 mg aspartame/kg bw/day from the 12th day of fetal life until natural death. The authors of the
2319 study reported a significant, dose-related increase in the incidence of malignant tumour bearing
2320 animals in males and a dose-related increased incidence in lymphomas/leukaemias in females, and an
2321 increased incidence of lymphomas/leukaemias in treated males and females at the high-dose group.
2322 Furthermore, a dose-related increased incidence of mammary gland carcinomas, in females and a
2323 significantly higher incidence of these carcinomas in females exposed to the high-dose was reported
2324 (Soffritti *et al.*, 2007; Chiozzotto *et al.*, 2011).

2325 The two rat studies have already been evaluated by the former AFC Panel (EFSA, 2006) and the ANS
2326 Panel (EFSA, 2009a, 2009b), respectively. Both Panels considered that the two ERF rat studies had
2327 flaws that bring into question the validity of the findings, as interpreted by the ERF. In particular, the
2328 high background incidence of chronic inflammatory changes in the lungs and other vital organs and
2329 tissues, and the uncertainty regarding the correctness of the diagnoses of some tumour types were
2330 major confounding factors in the interpretation of the findings of the study. The ANS Panel also noted
2331 that the increase in incidence of mammary carcinomas was not considered indicative of a carcinogenic
2332 potential of aspartame since the incidence of mammary tumours in female rats is rather high and varies
2333 considerably between carcinogenicity studies. The ANS Panel noted that the only consistent findings
2334 reported by the authors in the two rat studies were an increased incidence of lymphomas/leukaemias in
2335 female rats and an increased incidence of lymphomas/leukaemias in treated males and females of the
2336 high-dose group (EFSA, 2009a). A full evaluation of the ERF studies has been published elsewhere
2337 (EFSA, 2006; EFSA, 2009a, 2009b). In the present evaluation, the ANS Panel noted that an NTP
2338 review of selected ERF studies (conducted between 1974 and 1993) had found evidence of high rates
2339 of infection in the animals at the ERF institute (NTP-EPA, 2011). Furthermore, the NTP evaluation
2340 included a review of original histopathological slides. The review noted that there was no formal

2341 quality assessment process for pathology diagnoses, and that in a significant number of cases the NTP
2342 reviewers did not confirm malignancy as diagnosed by ERF pathologists. In view of these
2343 methodological concerns, given the consistency of findings in the studies reviewed, the Panel
2344 considered that these would apply to other studies carried out at the same time, including the
2345 aspartame studies. The COC in 2006 also evaluated the Soffritti *et al.* (2006) study on aspartame
2346 (COC, 2006). In light of the limitation in the design of this study and the use of animals with a high
2347 infection rate, the COC considered that no valid conclusions could be derived from it (COC, 2006).

2348 In the mouse study (Soffritti *et al.*, 2010) groups of 62–122 male and female Swiss mice were treated
2349 with aspartame in the feed at doses of 0, 2000, 8000, 16 000 or 32 000 mg/kg feed, equivalent to 0,
2350 250, 1000, 2000 or 4000 mg/kg bw/day, from pre-natal life (12 days of gestation) until death. The
2351 authors of the study reported a dose-related increase in hepatocellular carcinomas in the two highest
2352 dose male mice groups, and an increase in the incidence of alveolar/bronchiolar carcinomas in males
2353 of the high-dose group, whereas no compound-attributed carcinogenic effects were reported in female
2354 mice at any of the doses tested.

2355 The ANS Panel (EFSA, 2011a) and EFSA (EFSA, 2011b) concluded that the hepatic and pulmonary
2356 tumour incidences reported by Soffritti *et al.* (2010) all fall within their own historical control ranges
2357 for spontaneous tumours. It was also noted that Swiss mice are known to have a high background
2358 incidence of spontaneous hepatic and pulmonary tumours (Prejean *et al.*, 1973; Fox *et al.*, 2006).

2359 Based on these data, the Panel concluded that the results of the studies performed by Soffritti *et al.*
2360 (2010) do not provide evidence for a carcinogenic effect of aspartame in mice.

2361 Since the evaluations by the SCF and JECFA, the US National Toxicology Program (NTP) has carried
2362 out several 9-month carcinogenicity studies with aspartame in genetically modified mouse models
2363 (NTP, 2005). Fifteen male and fifteen female transgenic mice/group of three strains (Tg.AC
2364 hemizygous, p53 haploinsufficient and Cdkn2a deficient) were exposed to aspartame in the diet at
2365 doses ranging from 0, 3.1 to 50 g/kg diet for 9 months. The high-dose tested was equivalent to 7660
2366 and 8180 mg aspartame/kg bw/day in males and in females, respectively (Tg.AC hemizygous); 7280
2367 and 9620 mg aspartame/kg bw/day in males and in females, respectively (p53 haploinsufficient); 7400
2368 and 9560 mg aspartame/kg bw/day in males and in females, respectively (Cdkn2a deficient) (NTP
2369 2005). According to the NTP, there was no evidence of treatment-related neoplastic or non-neoplastic
2370 lesions in any of these studies. The former AFC Panel evaluated these studies and agreed with this
2371 conclusion. The overall number of animals with primary neoplasms, with benign neoplasms or with
2372 malignant neoplasms in aspartame-exposed groups of both sexes was not significantly increased
2373 compared to the respective controls (EFSA, 2006). The Panel agreed with these conclusions.

2374 **3.2.5. Reproductive and developmental toxicity of aspartame**

2375 **3.2.5.1. Studies on reproductive and developmental toxicity received following a public call for** 2376 **data which were available at the time of the previous JECFA and SCF evaluations**

2377 In the reproductive and developmental toxicity studies evaluated, aspartame was either administered in
2378 the diet or given by gavage (rabbit studies only).

2379 **3.2.5.1.1. Reproductive studies**

2380 *Rats*

2381 A two-generation reproduction study was performed in Charles River albino rats (12 male and 24
2382 female animals per group) that received aspartame (assumed to be 100% pure) in the diet at dose
2383 levels of 0 (control), 2000 and 4000 mg/kg bw/day (E11, 1971). The results of this study were not
2384 reported in detail. Dietary administration was conducted through two parental generations and one-
2385 litter per generation. Parameters evaluated included survival, body weights, feed consumption,
2386 physical appearance, and behaviour of the parental generations; indices of fertility, gestation, live birth

2387 and lactation; the litter size, appearance, behaviour, body weights and growth of offspring; and results
2388 of gross necropsy on selected weanlings. All parameters were comparable between the control and test
2389 groups with the exception of body weight suppression at weaning among the high-dose pups which
2390 was evident during both the first (F1) and second (F2) generation litters. Statistical analysis performed
2391 on the F1 and F2 litter revealed the body weights at the end of weaning of both sexes at the high-dose
2392 level to be statistically significantly lower (F1: 41 g and 38 g in males and females, respectively; F2:
2393 43 g and 41 g, in males and females, respectively) than those of the controls (F1: 48 g for both sexes;
2394 F2: 52 g for both sexes). Growth among the low-dose pups was comparable to the controls during both
2395 reproduction phases. The high-dose pups were noted to be generally smaller than the control and low-
2396 dose pups. The effect on haematological and biochemical parameters and on tissue morphology was
2397 examined in F2 pups from 1 up to 21 days of age. The results of this part of the study were described
2398 in more detail in another report below (E9, 1972).

2399 Forty pups, 20 males and 20 females (of the F2 generation from the study described above), offspring
2400 from the maternal groups that received aspartame at dose levels of 0 (control), 2000, or 4000 mg/kg
2401 bw/day, were used (E9, 1972). At 24 hours and at 5, 15, and 21 days postpartum, 10 pups from each
2402 group (5 pups/sex) were sacrificed and haematology, clinical chemistry and histopathology of selected
2403 tissues were performed. The appearance and behaviour of the pups during the study was comparable
2404 between control and test groups. Results of haematology and clinical chemistry (measured at days 5,
2405 15 and 21 postpartum) were unchanged at both dose levels. Microscopic examination of sections of
2406 heart, liver, stomach, kidney and urinary bladder revealed no apparent compound-related effects. In
2407 the kidney, minimal to slight hypertrophy and vesiculation of nuclei in cells of tubules in the inner
2408 cortex was noted primarily in the 15- and 21-day 4000 mg/kg bw/day males and females. Minimal
2409 changes were also seen in two 21-day 2000 mg/kg bw/day females. The authors noted that features of
2410 this same condition were also observed in a 15-day control male, but microscopic examination of renal
2411 tissue from 28-30-day-old weanlings revealed that such changes were not present in either control or
2412 treated rats of this age. The authors concluded that these changes were treatment-related but of a
2413 transient nature. A treatment-related effect at 2000 mg/kg bw/day at 21 days was equivocal, since the
2414 control group was not negative throughout. Such changes were not present in the treated rat at 28-30
2415 days of age. The Panel noted that the histological changes observed in the kidney seemed to be
2416 consistent with normal histological processes during postnatal development. There was evidence of a
2417 treatment-related effect at 15 and 21 days of age, but this was transient and appeared to have resolved
2418 by 28-30 days of age. The Panel identified a NOAEL from these studies of 2000 mg/kg bw/day based
2419 on the lower pup weights at weaning in both generations (E9, 1972; E11, 1971).

2420 In study E10 (1972), aspartame (DKP content 0.3%) (0, 2000 or 4000 mg/kg bw/day) was
2421 administered in the diet to Charles River rats (12 males and 48 females in the control group and 40
2422 females in the aspartame groups) for 64 days (males) or 14 days (females) prior to mating followed by
2423 a further dosing period until the end of lactation (female rats). During the pre-mating period, rats in the
2424 male groups ingested a mean daily dose of 1950 and 3867 mg/kg bw, respectively. In females the
2425 mean daily doses were 2017 and 4430 mg/kg bw during the pre-mating period, 1988 and 4100 mg/kg
2426 bw/ during gestation, 2375 and 4925 mg/kg bw during the first 14 days of lactation and 2700 and 5900
2427 mg/kg bw during the last 14 days of lactation. Aspartame had no significant effect on maternal sexual
2428 behaviour or fertility. Female body weights were slightly but significantly decreased in the high-dose
2429 group at pre-mating day 7, GD 18 and postpartum day 7. The authors also reported that the pups from
2430 aspartame-treated groups did not differ from controls in growth, survival or subsequent reproductive
2431 performance. The Panel agreed with this conclusion and identified a NOAEL of 4000 mg/kg bw/day
2432 (actual ingested doses ranged from 4100 mg/kg bw/day during gestation to 5900 mg/kg bw/day during
2433 the last 14 days of lactation), this being the highest dose level tested.

2434 In study E39 (1973), the effect of aspartame on peri- and post-natal development was studied in
2435 female rats (30 animals per dose group). Aspartame (with a content of DKP of 0.5%) was
2436 administered in the diet at the intended dose levels of 0 (control), 2000 or 4000 mg/kg bw/day. The
2437 recorded dose levels were 0, 2000 or 4000 mg/kg bw/day during gestation, increasing to 0, 3500 and
2438 6800 mg/kg bw/day during lactation. The number of viable pups per litter at birth and pup survival

2439 until weaning was significantly decreased in the high-dose group. Despite the reduced number of pups
2440 no effect on pup body weight (through to postpartum day 21) was recorded. There was no difference
2441 in feed consumption between the groups throughout the experiment. Maternal body weights were
2442 comparable between control and treated groups during gestation, except for GD 21, where the low-
2443 and high-dose groups showed a 5 and 9% reduction in body weight, respectively. During lactation,
2444 maternal body weights were comparable between the control and low-dose groups, whereas in the
2445 high-dose group mean body weight was significantly lower at postpartum day 21 (approximately 12
2446 %). According to the authors, the NOAEL in this study was 2000 mg/kg bw/day (recorded doses were
2447 2000 mg/kg bw/day during gestation and 3500 mg/kg bw/day during lactation). The Panel agreed with
2448 the author's conclusion on the NOAEL but noted that considering the poor survival of control pups,
2449 the health status of all the animals in the study might have been compromised.

2450 *Monkeys*

2451 A report summarising fragmentary data from a monkey study with aspartame was also submitted to
2452 EFSA following the public call for data (E88, 1975). The incompleteness of the data, however,
2453 severely limited the usefulness of this study. In summary, administration of aspartame to eight
2454 pregnant monkeys at dose levels up to 3800 mg/kg bw/day did not alter maternal appetite or body
2455 weight and did not induce seizure. There were no malformations observed in the fetuses at term. In
2456 addition, plasma phenylalanine levels in pregnant monkeys were not significantly increased, and the
2457 two abortions reported were spontaneous. However, no information on the reproductive capabilities,
2458 the reproductive status at the time of mating, the identity and reproductive history of the mate,
2459 evidence of actual establishment of the pregnancy or assessment of the gestational status of pregnant
2460 animals or experimental conditions of the animals were provided. The Panel concluded that the study
2461 provided insufficient information to evaluate reproductive and developmental effects of aspartame in
2462 monkeys.

2463 **3.2.5.1.2. Developmental studies**

2464 *Mice*

2465 The developmental toxicity of aspartame was investigated in Charles River CD-1 mice (E89, 1975)
2466 (Annex I). Aspartame (DKP content 0.29%) was administered in the diet to mated mice (four groups
2467 of 36 pregnant female) for 10 days from GD 6 to 15 at the intended dose levels equating to 0 (control),
2468 1000 (1400)²⁵, 2000 (2700), 4000 (5700) mg/kg bw/day. On GD 18, all females were sacrificed and
2469 necropsied. Fetuses were examined for external, soft tissue and skeletal abnormalities. The treatment
2470 had no effect on maternal survival and conception rates, maternal body weight changes, feed
2471 consumption, incidence of abortions and premature deliveries, proportion of litters that consisted of
2472 fetuses only, of both fetuses and resorption sites, or of resorption sites only, mean litter size, mean
2473 number of resorptions per litter, mean male and female fetal body weights and crown-rump distances,
2474 and the number of major malformations and skeletal variants, when compared to controls on a fetal
2475 incidence and a litter incidence basis. According to the Panel, the NOAEL for developmental toxicity
2476 in this study was 5700 mg/kg bw/day, the highest dose level tested.

2477 *Rats*

2478 Three segment III studies were performed in rats (peri- and post-natal development: E47, 1973; E48,
2479 1973; E 49, 1973).

2480 In two studies on peri- and post-natal development (E47, 1973 and E48, 1973), aspartame (DKP
2481 content 0.2-0.5%, respectively) was administered to rats (24 female rats per group in E47 and 30
2482 female rats per group in E48) at the intended doses of 0, 2000 or 4000 mg/kg bw/day. In E47, the rats
2483 received 2500 and 4400 mg/kg bw/day during gestation and 3600 and 6800 mg/kg bw/day during

²⁵ The doses in brackets refer to the actual average doses to which the animals were exposed to based on feed intake.

2484 lactation, whereas in E48, the actual doses were 1800 and 4000 mg/kg bw/day during gestation and
2485 3700 and 7000 mg/kg bw/day during lactation. The authors reported significant body weight
2486 suppression of the pups at birth at the high-dose (E47, E48) and weaning (at postpartum day 21) in
2487 females at both low- and high-doses (E47, E48) and in males at the high-dose (E48), significantly
2488 decreased survival at weaning at the high-dose (E47, E48) and incomplete eyelid opening in 5/164
2489 pups (E47) and 2/79 pups (E48) at the high-dose. The mothers exposed to the high-dose showed
2490 decreased feed intake at postpartum day 21 and reduced body weight at postpartum day 21 and during
2491 lactation (5-16%). Based on the results reported in these studies, the Panel identified a NOAEL of
2492 2000 mg/kg bw/day (E47:2500 mg/kg bw/day during gestation, 3600 mg/kg bw/day during lactation;
2493 E48: 1800 mg/kg bw/day during gestation, 3700 mg/kg bw/day during lactation).

2494 In a further segment III study (E49, 1973) in rats (30 females per group), the effects of aspartame
2495 (DKP content 0.5%) (0 or 4000 mg/kg bw/day in the diet) on peri- and post-natal development were
2496 compared with those of L-phenylalanine (1800 mg/kg bw/day) or L-aspartic acid (1700 mg/kg
2497 bw/day) or the combination of L-phenylalanine and L-aspartic acid (2100 and 1800 mg/kg bw/day).
2498 The actual doses that the rats received were 4000 mg aspartame/kg bw/day, 1800 mg L-
2499 phenylalanine/kg bw/day, 1700 mg L-aspartic acid/kg bw/day or 2100 + 1800 mg L-phenylalanine +
2500 L-aspartic acid/kg bw/day during gestation and 7800 mg aspartame/kg bw/day, 4200 mg L-
2501 phenylalanine/kg bw/day, 4000 mg L-aspartic acid/kg bw/day or 4600 + 3900 mg L-phenylalanine +
2502 L-aspartic acid/kg bw/day during lactation. The authors of the study reported that feed consumption
2503 was comparable between all groups but noted a significant decrease in body weight in the aspartame,
2504 L-phenylalanine and L-phenylalanine + L-aspartic acid groups as compared to the control group at
2505 postpartum days 7, 14 and 21. For example, at postpartum day 21, mean maternal weights recorded
2506 were 369.2 ± 6.5 g for controls, 327.7 ± 5.8 g for the aspartame group, 318.4 ± 10.8 g for the L-
2507 phenylalanine group and 316.4 ± 9.6 g for the L-phenylalanine + L-aspartic acid group. No differences
2508 in litter size were found but at postpartum day 21, L-phenylalanine and L-phenylalanine + L-aspartic
2509 acid group pup weights were notably lower (28.4 ± 3.3 g and 28.0 ± 2.3 g for male pups and $26.9 \pm$
2510 3.1 g and 28.5 ± 2.3 g for female pups) (statistically not significant) and significantly decreased in the
2511 aspartame group (25.9 ± 1.5 for male pups and 25.9 ± 1.3 g for female pups) compared to controls
2512 (34.2 ± 2.5 g for male pups and 33.4 ± 2.0 g for female pups). Pup survival was comparable between
2513 all the groups except for the L-phenylalanine + L-aspartic acid group (30.7% versus 49.5% in
2514 controls) where it was significantly lower. The authors of the study concluded that L-phenylalanine on
2515 its own or in combination with aspartic acid decreased maternal and pup body weight, which
2516 reproduced the observed effects of aspartame on these endpoints. The Panel agreed with the author's
2517 conclusion but noted the poor survival of control pups.

2518 The developmental toxicity of aspartame (was evaluated in a segment II study using dietary
2519 administration to pregnant albino rats (30 animals per group) from GD 6 to 15 inclusive (E5, 1970).
2520 Dose levels were 0 (control), 2000 and 4000 mg/kg bw/day. Parameters evaluated included maternal
2521 mortality, body weight, feed consumption, necropsy findings of the reproductive organs, in utero litter
2522 size (viable fetuses), resorptions, non-viable fetuses and fetal sex distribution; fetal size, gross
2523 appearance and examination of the fetal visceral and skeletal systems. Maternal mortality, body
2524 weight changes and necropsy findings were comparable between both aspartame treated groups and
2525 the control. Mean feed consumption throughout gestation was comparable in the control and low-dose
2526 groups, but was decreased by 15% at the beginning of the treatment period at the high-dose level. Feed
2527 consumption in the latter group gradually recovered and reached control values by the time compound
2528 administration was discontinued. No evidence of treatment induced fetopathological effects was
2529 observed in the 47 treated litters (589 term fetuses) examined. The Panel identified a NOAEL of 4000
2530 mg aspartame/kg bw/day for developmental and maternal toxicity.

2531 The Panel considered that, the two-generation reproductive toxicity study (E11, 1971) was not
2532 reported according to current standards but taken together with the segment I study (E10, 1972) and
2533 segment III studies (E47, 1973; E48, 1973) the available data were sufficient to conclude on
2534 reproductive toxicity. In summary, the results of the reproductive and developmental toxicity studies

2535 in rats indicate NOAELs that ranged from 2000 mg aspartame/kg bw/day (E11, 1971; E39, 1973; E47,
2536 1973; E48, 1973) to 4000 mg/kg bw/day (E5, 1970; E9, 1972; E10, 1972).

2537 *Rabbits*

2538 In several studies aspartame was administered in the diet at dose levels of 0 (control), 2000, or 4000
2539 mg/kg bw/day (E53, 1973; E54, 1974; E55, 1973) or 0 (control), 1000, or 2000 mg/kg bw/day (E62,
2540 1973) from GD 6 to GD 17-19, or 0 (control), 1400, or 2400 mg/kg bw/day (E63, 1973) from GD 6 to
2541 GD 18. The Panel noted that the actual dose to which the rabbits were exposed did not exceed 1880
2542 mg/kg bw/day (range 1160-1880 mg/kg bw/day) when the intended dose was 4000 mg/kg bw/day
2543 (administered as 4.8% (w/w) aspartame in the diet). This was a result of the considerable decrease in
2544 feed intake observed in the pregnant rabbits (32-62% decrease) in this dose group. The decrease in
2545 feed intake was much less pronounced in the 2000 mg/kg bw/day groups than in the 4000 mg/kg
2546 bw/day groups, as such the Panel noted that the actual aspartame doses in the low- and high-
2547 aspartame dose groups were often comparable.

2548 For example in E54 (1974), aspartame (DKP content 0.1%) was administered in the diet from GD 6 to
2549 19 to 16 pregnant female rabbits per dose group. The actual aspartame doses were reported to be 1880
2550 and 1870 mg/kg bw/day for the intended 2000 and 4000 mg/kg bw/day groups, respectively. The
2551 Panel noted that a significant decrease in fetal body weight and skeletal anomalies were reported for
2552 the 4000 mg/kg bw/day group but not for the 2000 mg/kg bw/day group even though both groups
2553 received the same dose of aspartame based on feed intake. Thus the Panel concluded from these
2554 observations that the developmental effects on body weight and skeletal development reported in the
2555 aspartame feeding studies may be caused by the significant depression of feed consumption in the
2556 high-dose group.

2557 In study E63 (1973) artificially inseminated rabbits (n=26 per group) received aspartame (with a DKP
2558 content of 1-4% as analysed in the diets) in a commercially-prepared pellet diet at a level of 3.4 or
2559 6.7% w/w from GD 6 to 18 resulting in a dose of 1400 and 2400 mg/kg bw/day. The control group
2560 was pair-fed (GD 6 to 24). There were no deaths in the pair-fed control group, two deaths in the 1400
2561 mg/kg bw group and three deaths in the 2400 mg/kg bw group. One female in the high-dose group had
2562 an early delivery. Conception rates were 96, 81 and 77% in the control, 1400 and 2400 mg/kg bw
2563 groups respectively. During gestation (GD 6 to 24), there were similar increases in body weight (9 and
2564 7%) in both aspartame-treated groups and an increase of 3% in the pair-fed controls. Increases in body
2565 weight during the entire gestation period were 5, 2, and 2% in these same groups. The feed
2566 consumption during treatment and post treatment as compared to pre-treatment, decreased by 6 and
2567 19%, respectively for the 1400 mg/kg bw group, increased by 3 and 2%, respectively for the 2400
2568 mg/kg group and decreased by 21 and 33%, respectively for the pair-fed control. The Panel noted that
2569 the feed consumption was lower in the pair-fed controls than in the treated animals. Uterine and litter
2570 data for the pregnant females sacrificed at term showed no evidence of compound-related effect. All
2571 fetuses were examined for external abnormalities and half of the fetuses were examined for skeletal or
2572 visceral examination. The total incidence of major malformations was 5 of 153 fetuses (3 of 21 litters)
2573 in the pair-fed control group, 2 of 100 fetuses (2 of 13 litters) in the 1400 mg/kg bw group and 4 of
2574 121 fetuses (3 of 17 litters) in the 2400 mg/kg group. The Panel considered the dose of 2400 mg
2575 aspartame/kg bw/day as a NOAEL, the highest dose tested.

2576 In another series of pre-natal developmental studies, rabbits were exposed to aspartame (at doses up to
2577 2000 mg/kg bw/day) using administration by gavage from GD 6 to 18. Most of these studies were
2578 confounded by poor health of the animals, and, in many cases, by a number of deaths in the treated
2579 groups, possibly related to the misdosing (E51, 1973; E52, 1973; E79, 1974).

2580 In study E51 (1973), artificially inseminated rabbits (36) received a daily dose of 2000 mg
2581 aspartame/kg bw/day (with a content of DKP of 1%) suspended in an aqueous solution of Tween-80
2582 and methylcellulose by gavage twice daily as equal doses from GD 6 through 18. Twelve control
2583 rabbits received the equivalent volume of vehicle as the treated animals. The study was confounded by

2584 poor health of the animals and the gavage technique issues. As a result, maternal mortality was high.
2585 The administration of aspartame was associated with depression of feed consumption by up to 40%.
2586 The controls were pair-fed to match the aspartame-treated animals with the lowest feed intake, but
2587 feed intake was noted to be lower still in control animals. The authors concluded that no embryotoxic
2588 or teratogenic effects were observed.

2589 Study E52 (1973) also suffered from maternal mortality due to poor health and the gavage technique
2590 issues. The study involved 24 control and 72 treated animals receiving a daily dose of 2000 mg
2591 aspartame/kg bw/day (DKP content up to 2%) suspended in an aqueous solution of Tween-80 and
2592 methylcellulose that was administered twice daily as equal doses. The control rabbits received the
2593 equivalent volume of vehicle. The control animals were pair-fed to compensate for the decrease in
2594 feed intake caused by aspartame. The control animals were pair-fed to match the aspartame-treated
2595 animals with the lowest feed intake, but feed intake was noted to be lower still in control animals. The
2596 authors concluded that no embryotoxic or teratogenic effects were observed.

2597 In study E79 (1974) artificially inseminated rabbits received a daily dose of 750 (37 animals) or 2000
2598 mg aspartame/kg bw/day (with a content of DKP up to 2%) (95 animals) in suspension in an aqueous
2599 solution of Tween-80 and methylcellulose and administered by gavage twice daily as equal doses to
2600 artificially inseminated rabbits from post-insemination day 6 through day 18. Control rabbits (32
2601 animals) received the same volume of vehicle as the treated animals. The study was confounded by
2602 poor health of the animals and the misdosing. As a result, maternal mortality was high. The
2603 administration of aspartame was associated with decreased feed consumption by up to 36%. The
2604 control animals were pair-fed to match the aspartame-treated animals with the lowest feed intake.
2605 However, especially aspartame-treated animals with a very restricted feed consumption died and the
2606 aspartame-treated animals with a more normal food consumption survived. As a result, the food
2607 consumption in the pair-fed control animals was noted to be lower than the food consumption of the
2608 aspartame-treated animals. The authors concluded that no embryotoxic or teratogenic effects were
2609 observed. However, the Panel considered the study not to be adequate to reach such a conclusion.

2610 Overall, the Panel considered that the data from the studies described above were confounded by the
2611 decrease in feed intake (when aspartame was administered via the diet or by gavage), the poor health
2612 of the animals, and, in many cases, by a number of deaths of pregnant rabbits in the treated groups
2613 most possibly related to misdosing.

2614 In a separate study in pregnant rabbits (E90, 1975), which contained a sufficient number of animals for
2615 the evaluation of developmental toxicity, aspartame (DKP content 0.29%) suspended in an aqueous
2616 solution of Tween-80 and methylcellulose was administered by gavage twice daily to artificially
2617 inseminated rabbits from GD 6 up to 18. The doses of aspartame were 0 (control), 500, 1000 and 2000
2618 mg/kg bw/day. The controls received the same volume of vehicle per kg bw as the treated animals.
2619 The study included two additional groups, one that received L-phenylalanine (820 mg/kg bw/day) and
2620 one that received L-aspartic acid (1100 mg/kg bw/day), which, on a molar basis, were levels
2621 equivalent to 75% and 134% of the amount of the same amino acids theoretically available from 2000
2622 mg/kg bw aspartame. Each treatment group consisted of 50 inseminated rabbits. On GD 28, all
2623 surviving females were sacrificed and necropsied. Fetuses were removed by hysterectomy and
2624 examined, weighed, measured and preserved for either soft tissue or skeletal examination. Parameters
2625 evaluated included: maternal survival rates; maternal conception rates; incidences of abortion or of
2626 premature delivery; maternal body weights, feed consumption incidences of litters consisting of
2627 fetuses only, of both fetuses and resorptions, or of resorptions only; mean litter sizes; mean number of
2628 resorption sites per litter; mean fetal body weights and crown-rump distances; incidences of major and
2629 minor malformations on a per litter or per fetus basis. A number of animals died spontaneously during
2630 the study (4/control; 4/500 mg/kg bw/day; 1/1000 mg/kg bw/day; 4/2000 mg/kg bw/day;
2631 5/phenylalanine; 2/aspartic acid), mainly due to misdosing. Eight control females, 4 low- and mid-
2632 dose females and 13 high-dose females were non-pregnant. No abortions were detected in the control,
2633 mid-dose and L-aspartic acid groups, two abortions in the low-dose aspartame group and 24 abortions
2634 were observed in the high-dose aspartame group (a significant increase compared to controls) and four

2635 in the L-phenylalanine group. An approximately 65% decrease in mean feed consumption was
2636 observed in the 2000 mg/kg bw/day group animals, beginning on the first day of treatment (GD 6) and
2637 continuing throughout the treatment period (GD 18). Similarly, feed consumption for the L-
2638 phenylalanine group females from gestation days 6 through 18 was significantly decreased by
2639 approximately 40% as compared to the controls. The decrease in feed consumption in the high-dose
2640 aspartame group was followed by body weight loss, first apparent on day 10. These body weight
2641 losses were statistically significant ($P < 0.05$) in the high-dose group beginning on GD 13, and were
2642 more marked in those females that ultimately aborted, which comprised well over one-half of the
2643 pregnant high-dose females. Feed consumption was lower in those high-dose animals that
2644 subsequently aborted than in those that maintained their pregnancies to GD 28. In view of the
2645 statistically significant decrease in feed consumption and the high-incidence of abortions, the authors
2646 of the report subdivided the data from the high-dose animals into two groups for further analysis: those
2647 from animals surviving until sacrificed on gestation day 28 and retaining their litter (no abortions); and
2648 those from animals surviving until day 28 but aborting their litter. This analysis showed that females
2649 in the high-dose group having aborted had statistically significantly lower body weights ($p < 0.05$)
2650 compared to controls and to non-aborting females, starting at gestation days 13 and 18, respectively.
2651 Females in the high-dose group having retained their litter (non-aborting) did not show any
2652 statistically significant difference in body weight as compared to controls or to aborting females
2653 during the entire duration of the study (28 days). Since the decrease in body weight started several
2654 days before (13 and 18 days) the abortions (28 days), the authors concluded that abortion was a
2655 consequence of significant and rapid body weight loss caused by decreased feed consumption.

2656 At Caesarean section, only 11 pregnant females of the high-dose group were available for evaluation
2657 versus 36 control, 41 low-dose and 44 mid-dose pregnant females. The mean number of resorptions in
2658 the high-dose group was increased although not statistically significant due to the low numbers and
2659 large variability between these litters. However, the mean number of resorptions was significantly ($P <$
2660 0.05) increased in the L-phenylalanine group. Mean fetal body weight and length were significantly
2661 reduced in both the 2000 mg/kg bw/day group and the L-phenylalanine group (mean body weights (in
2662 g) for male fetuses 23.5 (aspartame) and 28.2 (L-phenylalanine) vs 33.2 in controls; for female fetuses
2663 23.3 (aspartame) and 27.1 (phenylalanine) vs 32.0 in controls). The decrease was approximately
2664 proportional to the reduction in feed consumption. The number of tarsal and metacarpal ossification
2665 centres, an indicator of normal fetal growth, was significantly ($p < 0.05$) reduced in the rabbit fetuses
2666 of the 2000 mg/kg bw/day and phenylalanine groups. Additionally, a significant increase in the
2667 incidence of an extra (13th) pair of ribs and a reduction in sternbral ossification centres were observed
2668 in high-dose aspartame group. Comparisons based on either fetal incidence or litter incidence revealed
2669 a significantly higher rate of total (major and minor) malformations in the 2000 mg/kg bw/day group
2670 animals as compared to the concurrent control group. The increases in major malformations were due
2671 primarily to three instances of cleft palate in two litters in the high-dose aspartame group and five
2672 instances of umbilical hernia or omphalocele in five separate litters in the L-phenylalanine group. The
2673 increases in minor malformations were due in large part to various vertebral defects and fused or split
2674 sternbrae. The authors indicated that all the above reported effects, observed at 2000 mg/kg bw/day
2675 and to a lesser extent in the L-phenylalanine group, appeared to be produced by a severe reduction of
2676 nutrient intake.

2677 The Panel considered the explanation given by the authors as plausible as rabbits are known to be
2678 susceptible to gastrointestinal tract disturbances following administration of high doses of test
2679 preparation by gavage, resulting in lower feed intake and even disturbed clinical appearance, death and
2680 abortions.

2681 Matsuzawa *et al.* (1981) reported that, pregnant rabbits restricted to 20 or 60 g feed/day from gestation
2682 days 6 to 20 showed statistical significantly higher fetal losses than pregnant rabbits fed 150 g
2683 feed/day and control group.

2684 Cappon *et al.* (2005) studied the effects of feed restriction on reproductive performance and
2685 development of the fetuses in rabbits. Groups of 15 New Zealand White rabbits were offered 150

2686 (control), 110, 75, 55, 35, and 15 g feed/day from GD 7-19. An increased number of abortions
2687 associated with an impairment of body weight gain were observed after feed restriction to 15 g
2688 feed/day. Feed restriction to 75 g feed/day was associated with reduced ossification of the fetal
2689 skeleton whereas reduced fetal weight and increased incidence of fetuses with reduced ossification of
2690 the skeleton were noted at feed consumption of less 75 g feed/day. Cappon reported no association of
2691 fetal malformation with up to 90% feed restriction. The author concluded that the weight-of-evidence
2692 suggested that restricting rabbit feed during pregnancy, and the associated impairment of maternal
2693 gestational body weight gain could cause abortion, and fetal growth retardation exhibited by reduced
2694 fetal body weight and reduced skeletal ossification, but induced, remarkably, few (or no)
2695 malformations.

2696 The Panel further noted that the gastrointestinal effects in the rabbit may be species specific and would
2697 therefore not be relevant for humans. Although aborting female rabbits from the high-dose group in
2698 study E90 (1975) manifested body weight loss and lower, to very restricted, feed consumption
2699 compared to those that maintained their pregnancies, the Panel was unable to determine that the
2700 reported effects on maternal organism and fetus were due to a reduced nutrient intake, as suggested by
2701 the authors. The Panel also considered the possibility that the high phenylalanine concentration
2702 resulting from metabolism of aspartame was at least in part responsible for the effects in the high-dose
2703 aspartame group because similar effects, although less severe, were seen in the phenylalanine group.
2704 However, the Panel noted that the dose of L-phenylalanine administered was 25% less than the
2705 animals would have received as L-phenylalanine from the high-dose of 2000 mg aspartame/kg
2706 bw/day.

2707 Based on the above considerations the Panel identified a NOAEL of 1000 mg aspartame/kg bw/day
2708 for maternal and developmental toxicity in study E90 (1975).

2709 In summary, the highest NOAEL for developmental toxicity in rabbits of 2400 mg aspartame/kg
2710 bw/day was identified by the Panel in study E63 (1973), in which the test compound was administered
2711 in the diet. The NOAEL in the study in rabbits dosed by gavage (E90) was 1000 mg aspartame/kg
2712 bw/day, based on maternal toxicity in the 2000 mg/kg bw/day group, which was accompanied by a
2713 severe decrease in mean feed intake. The Panel noted that the severely decreased feed intake could
2714 have a negative effect on the nutritional status of these females and the observed adverse effects could
2715 result from gastrointestinal disturbances as well as from the potential effect of phenylalanine.

2716 The overview of the reproduction and developmental studies is presented in Annex I.

2717 **3.2.5.2. Additional studies on reproductive and developmental toxicity**

2718 **3.2.5.2.1. Reproductive studies**

2719 *Rats*

2720 The effect of aspartame on reproduction was investigated in Sprague Dawley rats by Brunner *et al.*
2721 (1979). Rats (total number not stated) were fed diets containing 0, 2, 4 or 6% aspartame (w/w) during
2722 pre-breeding, gestation and lactation and post-weaning. Feed consumption measurements suggest that
2723 the animals were exposed to doses of aspartame of approximately 0, 1600, 3500 and 5000 mg/kg
2724 bw/day during pre-breeding and gestation, 0, 4000, 7000 and 9600 mg/kg bw/day during lactation and
2725 0, 3000, 6000 and 9000 mg/kg bw/day post-weaning. Another group of rats received phenylalanine in
2726 the diet (3%) (approximately 2500 mg/kg bw/day during pre-breeding and gestation, 4600 mg/kg
2727 bw/day during lactation). Breeding pairs were enrolled in the study until 18 litters were available for
2728 each group. There was no effect on pre-breeding body weights or on maternal weight gain during
2729 gestation, but rats exposed to the highest aspartame dose during lactation lost more weight than the
2730 other dietary groups (9% of decrease from Day 1 to Day 21). Increased offspring mortality was
2731 observed in rats fed the highest aspartame dose and in the phenylalanine-exposed animals. Gestation
2732 length and litter size were not affected by aspartame or phenylalanine; however, pups fed 6%
2733 aspartame weighed significantly less than controls after 30 days and remained lighter throughout the

2734 study. Eye opening was delayed by one day in pups in the 6% aspartame and 3% phenylalanine diet
2735 groups, but timing of pinnae detachment and incisor eruption were not affected. The Panel agreed with
2736 the conclusion of the authors and noted that the NOAEL was 4% in the diet, corresponding to 3500
2737 mg/kg bw/day during gestation.

2738 Potential post-coital fertility was assessed in Charles River rats (6 animals/group) following
2739 administration of 300 mg aspartame/kg bw/day in corn oil by gavage for seven days after mating
2740 (Lennon *et al.*, 1980). Controls received corn oil. There were no differences noted in the number of
2741 rats that became pregnant. The Panel noted that the number of animals used in these studies was small.

2742 *Hamsters*

2743 Potential post-coital fertility was also assessed in a similar experiment in female hamsters with a
2744 control group of 15 animals treated with corn oil and a group of five animals given daily doses of 300
2745 mg aspartame/kg bw in corn oil by gavage for seven days after mating (Lennon *et al.*, 1980). No
2746 differences were reported in implantation and regression of corpora lutea. The Panel noted that the
2747 number of animals used in these studies was small.

2748 **3.2.5.2.2. Developmental studies**

2749 *Mice*

2750 Mahalik and Gautieri (1984) reported a study in pregnant CF-1 mice (number not reported) which
2751 were administered aspartame by gavage at doses of 1000 mg/kg bw/day and 4000 mg/kg bw/day from
2752 GD 15 to 18. There were no significant differences between control and treated animals in negative
2753 geotaxis, surface or air righting. However, the achievement age for visual placing was significantly
2754 delayed, in a dose-dependent manner, in both groups of treated animals (22 ± 0.7 at 1000 mg/kg
2755 bw/day and 24.4 ± 0.7 at 4000 mg/kg bw/day *vs* 20 ± 0.9 in controls). The Panel noted that
2756 uncontrolled litter size and pup weight were not taken into consideration and performance of the
2757 offspring was assessed only the last day of achievement for the entire litter.

2758 In a similar study by McAnulty *et al.* (1989), CF-1 pregnant mice (20/group) were administered
2759 aspartame by gavage at doses of 0, 500, 1000, 2000, and 4000 mg/kg bw/day from GD 15 to 18.
2760 Maternal body weight, feed consumption, gestation length, reproductive indices, and litter size were
2761 not affected by the treatment. In the pups, there were no changes in body weights, negative geotaxis,
2762 surface and midair righting reflexes. In addition, reflex pupil closure, and ophthalmoscopic
2763 examination in the offspring were not altered by the treatment. Time of eye opening was statistically
2764 significantly later than control at the lowest and highest doses (14.3 ± 0.15 for both doses *vs* $14.8 \pm$
2765 0.15 in controls), as well as the development of the visual placing which was statistically significantly
2766 lower than control at the lowest dose of 500 mg aspartame/kg bw/day (18.8 ± 0.28 *vs* 20.5 ± 0.40 in
2767 controls). The authors considered these findings as isolated points that may vary in either direction.
2768 They were not dose-related and as such, the results were not considered to be biologically meaningful.
2769 The authors concluded that *in utero* exposure to aspartame in CF-1 mice did not affect the physical
2770 and functional development of the visual system of the pups. The Panel agreed with the conclusion of
2771 the authors.

2772 *Rats*

2773 Holder (1989) studied the effects of aspartame administered in the drinking water to rats from 12 days
2774 prior to conception until the pups were 38 days old. Adult Sprague-Dawley rats (10/group) were given
2775 either water or water containing aspartame (0.007%, 0.036%, 0.18% or 0.9% w/v) or phenylalanine
2776 (0.45% w/v). The authors reported that adult rats consumed an average of 14, 68, 347, and 1614 mg/kg
2777 bw/day of aspartame respectively, and 835 mg/kg bw/day of phenylalanine. After weaning, the pups
2778 (4/sex/litter) were given the same treatment as the adult rats, and consumed an average of 32, 154,
2779 836, and 3566 mg/kg bw/day of aspartame respectively, and 1795 mg/kg bw/day of phenylalanine.
2780 The two parameters of morphological development, latency of pinnae detachment and eye opening, as

2781 well as the two parameters of reflex development, latencies for surface righting reflex (which
2782 decreased in a dose-dependent manner with aspartame treatment) and negative geotaxis were not
2783 statistically significantly affected by aspartame or phenylalanine. There were no differences on the
2784 performance of spatial memory in the radial-arm maze or in the milk maze. Moreover, the latency of
2785 mothers to retrieve their pups was not affected by either treatment. The authors concluded that
2786 exposure in utero and later directly of the pups did not affect reflex development, morphological
2787 development and spatial memory. The Panel agreed with the conclusion of the authors.

2788 *Monkeys*

2789 In a postnatal developmental study, monkeys were fed with 0, 1000, 2000, or 2500-2700 mg
2790 aspartame/kg bw/day for 9 months. No effects on body weight gain, crown-heel length, teething,
2791 vocalization, alertness, or general behaviour were observed. No effects on haematological, plasma
2792 chemistry and urinalysis parameters or on ECG were observed. Aspartame exposure had no effects on
2793 learning performance and hearing ability. The NOAEL was 2500-2700 mg aspartame/kg bw/day, the
2794 highest dose of aspartame tested (NTP-CERHR Report, 2003).

2795 **3.2.5.2.3. Other studies**

2796 *Mice*

2797 Collison *et al.* (2012a) investigated the effects of neonatal exposure to monosodium glutamate (MSG),
2798 aspartame or a combination of both, on glucose and insulin homeostasis in C57BL/6J mice chronically
2799 exposed to these food additives starting *in utero*. Mouse offspring (12 to 18/sex/group) were bred,
2800 weaned and maintained on the following diet groups for 17 weeks: standard diet (control), standard
2801 diet with MSG (120 mg/kg bw/day) alone, standard diet with aspartame (approximately 50 mg/kg
2802 bw/day) alone, standard diet with MSG and aspartame (at the same doses as when the compounds
2803 were administered separately). Aspartame alone caused a 1.6-fold increase in fasting blood glucose
2804 and reduced insulin sensitivity. MSG alone decreased blood triglyceride and total cholesterol levels.
2805 The combination of MSG and aspartame increased body weight, and caused a further 2.3-fold increase
2806 in fasting blood glucose compared to control diets. Total cholesterol levels were reduced in both
2807 aspartame-containing diets in both sexes. A positive correlation between aspartame intake and body
2808 weight at 6 weeks and 17 weeks, and aspartame intake and fasting blood glucose levels at 17 weeks
2809 was also noted. The authors concluded that aspartame exposure might promote hyperglycaemia and
2810 insulin intolerance, and MSG might interact with aspartame to impair further glucose homeostasis.

2811 The authors attributed indirectly the increases in fasting blood glucose and reduced insulin sensitivity
2812 observed in their study to an elevated level of aspartame-derived phenylalanine that could potentially
2813 accumulate in the brain leading to changes in the regional brain concentrations of neurotransmitters
2814 and a dose-dependent reduction in dopamine, serotonin and noradrenalin levels. The Panel noted that
2815 the mouse strain used in this study is known to harbour a dominant trait showing a high susceptibility
2816 to diet-induced type-2 diabetes, as well as obesity and atherosclerosis (JAX Mice Database). The
2817 Panel also noted that no dose-response was assessed in this study and that other authors (Berglund *et*
2818 *al.*, 2008²⁶) have reported higher insulin and glucose basal levels in the C57BL/6J mice strain
2819 suggesting that those parameters vary in that mice strain. The Panel also noted that phenylalanine and
2820 glutamate can also arise from the protein content of the diet and thus the actual contribution of
2821 aspartame to the total phenylalanine and glutamate amino acids pool should be clearly defined to be
2822 able to attribute exclusively an increment in fetal amino acid pool to aspartame intake. Finally, the
2823 Panel also observed that short term preliminary interventional trials undertaken in human volunteers
2824 suggest that test meals containing aspartame significantly reduce postprandial glucose levels and
2825 insulin levels compared to a test meal containing sucrose (Anton *et al.*, 2010).

2826 In another study Collison *et al.* (2012b) investigated the effects of neonatal exposure to aspartame,

2827 commencing in utero, on changes in blood glucose parameters, spatial learning and memory in
2828 C57BL/6J mice (numbers of animals not clearly reported but according to the tables published they
2829 are between 12 and 18 per group depending on the parameter measured) that were administered
2830 standard diet (control) or standard diet with aspartame (approximately 50 mg/kg bw/day) for 17
2831 weeks. Increased weight gain, increased fasting glucose levels and decreased insulin sensitivity was
2832 observed in treated male mice compared to controls. These effects were less apparent in females,
2833 which did however show significantly raised fasting glucose levels. The escape latencies used in the
2834 Morris Water Maze to test spatial learning and memory were consistently higher in treated male mice
2835 than in controls, as well as the thigmotactic behaviour and time spent floating directionless. Spatial
2836 learning of female-treated mice was not significantly different from controls. Reference memory was
2837 changed in both sexes, and the aspartame-fed mice spent significantly less time searching for the
2838 former location of the platform. Visceral fat deposition positively correlated with non-spatial search
2839 strategies (i.e. floating and thigmotaxis), and negatively with time spent in the target quadrant and
2840 swimming across the location of the escape platform. Overall, the authors of the study concluded that
2841 lifetime exposure to aspartame, commencing in utero, might affect spatial cognition and glucose
2842 homeostasis in C57BL/6J mice, particularly in males.

2843 The Panel noted that the selection method of pups for several tests was not clearly reported by
2844 Collison *et al.* and that only one dose was used rendering thus any assessment of dose-response
2845 relationship not possible.

2846 The Panel noted that the findings in mice reported by Collison *et al.* (2012 b) might not apply to other
2847 species, since in a large study on Sprague-Dawley rats (Holder, 1989) performances on radial-maze
2848 and milk maze was similar for rat pups given aspartame at doses from 14 to 1614 mg/kg bw/day or
2849 phenylalanine at a dose of 835 mg/kg bw/day compared to controls. These findings indicated to the
2850 authors that spatial memories, as well as motor and visual components of these tasks were not affected
2851 by perinatal exposure to aspartame.

2852 *Rats*

2853 Lennon *et al.* (1980) conducted a larger experiment with 60 female Charles River rats to assess the
2854 effect of aspartame in the diet on lactation. After mating and delivery of pups, the dams were allocated
2855 in body weight matched pairs to treatment groups for a pair-feeding experiment. Groups of six dams
2856 were fed diets containing 1, 2, 4, 7.5, or 14% aspartame by weight for 21 days (during the lactation
2857 period). Pair-matched controls were fed the same amount of feed as their match consumed ad libitum
2858 the previous day, but without added aspartame. Feed consumption and body weights were
2859 significantly lower in dams fed 7.5 and 14% aspartame diets on day one and throughout the
2860 experiment (e.g. body weight reduction by up to 65%). Similar body weight losses were observed in
2861 the pair-fed controls; therefore, the loss of body weight was considered by the Panel to be due to the
2862 lower feed consumption. Based on feed consumption data, the actual doses of aspartame by the
2863 different groups were 0, 1870, 3680, 7120, 9110 and 8830 mg/kg bw/day. Pup body weight was also
2864 significantly reduced in the 9110 and 8830 mg/kg bw/day groups. Pup survival was significantly
2865 reduced in the highest dose group and pair-matched controls. Dams fed the highest dose of aspartame
2866 and pair-matched controls had a higher incidence of resting or inactive mammary glands, which were
2867 attributed by the authors of the study to a lack of suckling pups and severe feed restriction. The Panel
2868 noted that there were no effects of aspartame at doses up to 7120 mg/kg bw/day on feed consumption,
2869 dam and pup body weight, pup survival and mammary gland histology. Therefore, the Panel identified
2870 a NOAEL of 7120 mg/kg bw/day.

2871 *Rabbits*

2872 Ranney *et al.* (1975) investigated the phenylalanine and tyrosine content of maternal and fetal body
2873 fluid in rabbits following dietary exposure to aspartame. Thirty pregnant New Zealand female rabbits
2874 were fed with either a standard diet (control) or a diet containing aspartame at a level of 6%, beginning
2875 from GD 6. Maternal and fetal blood samples and fetal amniotic fluid were taken up to GD 20.

2876 Maternal plasma phenylalanine and tyrosine levels significantly increased in aspartame-fed animals
 2877 compared to controls, reaching a peak of 4.9 ± 0.3 mg/dl (Phe, 296.6 μ M) and 9.9 ± 0.6 mg/dl (Tyr,
 2878 546 μ M) on GD 9. These values returned to normal on GD 20. Fetal plasma tyrosine was significantly
 2879 higher in aspartame-fed animals compared to controls, reaching a peak of 7.4 ± 0.2 mg/dl (408 μ M) on
 2880 GD 20, whereas phenylalanine was unchanged [2.5 ± 0.2 mg/dl (151.3 μ M) in controls vs 2.3 ± 0.2
 2881 mg/dl (139.2 μ M) in aspartame-treated rabbits]. This resulted in a significant drop in the Phe/Tyr ratio
 2882 in both maternal (up to 58%) and fetal plasma (53%). Phenylalanine and tyrosine concentrations in the
 2883 amniotic fluid were consistently higher in the aspartame -treated animals compare to controls at GD
 2884 16 and 20, with peaks at 20.4 ± 2.8 mg/dl (Phe, 1235 μ M) and 33.4 ± 2.0 mg/dl (Tyr, 1843 μ M) at GD
 2885 20. The ratios of amniotic fluid/maternal and fetal plasma phenylalanine and tyrosine concentrations at
 2886 GD 20 were higher in treated-animals compared to controls. Both phenylalanine and tyrosine
 2887 concentrations of the supernatants from whole fetal homogenates at GD 16 and 20 were higher in
 2888 treated-animals than in control, and, as a consequence, the ratio of Phe/Tyr was consistently lower
 2889 than control values. The authors concluded that the treatment of pregnant rabbits with a high-dose of
 2890 aspartame did not affect the transport of Phenylalanine and tyrosine across the placental membrane
 2891 since the ratios of fetal/maternal plasma amino acid concentrations were unaffected by the treatment.

2892 3.2.6. Other studies on aspartame

2893 3.2.6.1. Neurotoxicity

2894 In order to investigate whether aspartic acid generated from aspartame might induce neurotoxicity, a
 2895 study in neonatal mice was performed. Young, 6-10-day-old neonatal hybrid A/JAX-ICR mice (12-18
 2896 animals of both sexes per dose groups, sex ratio not specified) were dosed between PND 6 and 10 by
 2897 gavage with aspartame at single doses of 250 (n=12 out of 3 litters), 500 (n=19 out of 5 litters), 1000
 2898 (n=21 out of 3 litters), 1500 (n=17 out of 4 litters) or 2000 (n=5 out of 18 litters) mg/kg as a 10%
 2899 slurry (volume, vehicle and duration of exposure not stated) (E94, year not reported). Upon sacrifice,
 2900 brains were subjected to histopathological examination. The Panel however noted that the design of
 2901 the study was not adequately described and no control group was included, and, therefore, this study
 2902 was considered not relevant for the overall risk assessment.

2903 In another study, 6-12-day-old mice (sex and strain not specified) aspartame was dosed orally at 500
 2904 (27 animals), 1000 (15 animals), 1500 (17 animals) or 2000 (18 animals) mg aspartame/kg bw in a
 2905 10% (w/v) slurry (volume and vehicle not stated) (Reynolds *et al.*, 1976). The Panel noted the absence
 2906 of control animals in the study and therefore did not take this study into consideration.

2907 Long Evans weanling rats (21 day old, 32/sex/group) were given a standard diet (control) or standard
 2908 diet supplemented with L-phenylalanine (2.5% or 5%), or aspartame (4.5% or 9 %) for 13 weeks (E14,
 2909 1972). For aspartame in the diet, the calculated mean daily intakes of L-phenylalanine were: 3000
 2910 mg/kg bw/day (4.5% aspartame) and 6100 mg/kg bw/day (9% aspartame) in males, and 3100 mg/kg
 2911 bw/day (4.5% aspartame) and 6800 mg/kg bw/day (9% aspartame) in females. For L-phenylalanine in
 2912 the diet, the calculated mean daily intakes of L-phenylalanine were: 3000 mg/kg bw/day (2.5% L-
 2913 phenylalanine) and 6700 mg/kg bw/day (5% L-phenylalanine) in males, and 3200 mg/kg bw/day
 2914 (2.5% L-phenylalanine) and 6500 mg/kg bw/day (5% L-phenylalanine) in females. No differences in
 2915 physical conditions were noted between the groups. For the low dose groups (2.5% L-phenylalanine
 2916 and 4.5% aspartame), some effects were reported on occasion but they were transient and not dose-
 2917 related. For the high dose groups (5% phenylalanine and 9% aspartame), statistically significant
 2918 impaired learning performances were reported (i.e. conditioned or nondiscriminated avoidance
 2919 responses). The Panel concluded that L-phenylalanine administered at doses of 6000 mg/kg bw/day
 2920 for 90 days to rats, impaired learning performances. Doses of aspartame providing the same dose of L-
 2921 phenylalanine induced similar effects. At a lower dose (3000 mg L-phenylalanine/kg bw/day), the
 2922 effects were not reported.

2923 Male weanling Long-Evans rats (8 animals) were dosed with aspartame (1000 mg/L in drinking water;
 2924 actual intake not specified) for 14 weeks, after which terminal blood samples were taken for

2925 measurement of plasma glucose, immunoreactive insulin and leptin (Beck *et al.*, 2002). The study
2926 contained a control group of 16 rats that received tap water. Epididymal, perirenal and subcutaneous
2927 fat pads were dissected and the liver, spleen and heart were also taken. Frozen sections of brain (300
2928 μm) were cut and individual brain regions were sampled by micropunching for the analysis of
2929 neuropeptide Y. The authors of the study reported that feed and fluid intakes were similar between
2930 treated and control groups, but aspartame treated rats gained less body weight than controls, with fat
2931 pads weighing less than controls (34.86 ± 1.39 g vs 27.59 ± 1.89 g, $p < 0.02$). Whereas terminal plasma
2932 insulin and glucose levels did not differ between groups, the level of hypothalamic neuropeptide Y in
2933 the arcuate nucleus (but not in other parts of the brain) was significantly lower in aspartame-treated
2934 rats than in controls by 23.2% ($p < 0.02$). The author reported that the reasons for the neuropeptide Y
2935 decrease were not clear and further analysis of other neuropeptides in the arcuate nucleus plus further
2936 histological controls will contribute to elucidate if the observed effects are physiological or treatment-
2937 related. The panel agreed with these observations.

2938 Rabbits (3 months old) and Wistar rats (30 days old) were dosed with aspartame (2000 mg/kg bw/day
2939 in diet slurry) for 30 days (Puica *et al.*, 2008, 2009). No vehicle or sham-gavaged control group is
2940 mentioned and the frequency of dosing is not stated. At the end of 30 days of dosing, animals were
2941 decapitated and the brains prepared for EM. Ultrastructural damage, described as 'selective
2942 degeneration of all subcellular neurons ultrastructures both in CA1 pyramidal neurons of the
2943 hippocampus and ventromedial area of the hypothalamus' was reported in both species, and the
2944 authors conclude that juvenile rats and rabbits are particularly susceptible to neurotoxic effects
2945 induced by aspartame. However, the Panel noted that the interpretation of these studies was not
2946 possible because of the lack of experimental details, the absence of appropriate control animals and of
2947 statistical analysis of the data.

2948 The potential neurotoxic effects of high doses of aspartame were also examined in monkeys (E104,
2949 1979; Reynolds *et al.*, 1980; E105, year not reported). Macaques aged 1-22 days (species *Macaca*
2950 *mulatta*, *Macaca fascicularis* and *Macaca arctoides*) weighing 280-820 g were administered by
2951 gavage a single dose of either vehicle (2-5 ml water/animal; 2 males and 3 females), aspartame (2000
2952 mg/kg bw; 2 males and 6 females) or a combination of aspartame and monosodium glutamate (MSG)
2953 (2000 + 1000 mg/kg bw; 3 males and 3 females) as a 10% (w/v) slurry. For aspartame, the dose was
2954 calculated to correspond to 6-11 ml administered/animal. The Panel noted that this dosing volume was
2955 higher than what the controls received. The study also included untreated controls of five males and
2956 one female. Blood samples were taken up to 4-5 hours after dosing and the animals were then
2957 sacrificed. Light and electron microscopic examination of the brain sections revealed no significant
2958 abnormalities in response to aspartame or aspartame + MSG treatments. Measurements of plasma
2959 amino acid levels showed that aspartic acid reached a peak concentration of 230 ± 330 μM at 60 min
2960 ($p < 0.0001$) and that phenylalanine reached a peak concentration of 950 ± 590 μM at 90 min. Basal
2961 levels (0 minute time point) for aspartic acid and phenylalanine were 0.69 ± 0.43 μM and 5.93 ± 2.81
2962 μM , respectively. The Panel noted that the design of the study is inappropriate to evaluate effectively
2963 any potential neurotoxic effects of aspartame.

2964 The Panel is aware that the neurotoxicity of aspartame was also reviewed by Magnuson *et al.* (2007)
2965 and subsequently by the National Expert meeting of the EFSA Advisory Forum (EFSA, 2010a).
2966 Magnuson *et al.* (2007) concluded that the data obtained from the extensive investigations of the
2967 potential neurotoxic effects due to aspartame consumption, did not support the hypothesis that
2968 aspartame present in the human diet would cause any impairment of the neuronal function, learning or
2969 behaviour (Magnuson *et al.*, 2007). Likewise, the National Expert Meeting of the EFSA Advisory
2970 Forum (EFSA, 2010a) concluded that no new data were identified reporting a link between aspartame
2971 intake and enhanced susceptibility to seizures, behaviour, mood and cognitive function (EFSA,
2972 2010a). These conclusions were in line with the SCF conclusions in 2002 (SCF, 2002). The Panel
2973 agreed with their conclusions.

2974 **3.2.6.2. Studies on the effect of aspartame administration on xenobiotic metabolising enzymes**

2975 An early study (E15, 1972) indicated that the oral administration of aspartame to male Charles River
2976 rats (2000 – 4000 mg/kg) for four days had no acute effect on hepatic cytochrome P450 (CYP)-
2977 mediated xenobiotic metabolism, as measured as aminopyrine N-demethylation, p-nitroanisole N-
2978 demethylation, hexobarbital sleeping time or zoxazolamine paralysis time. Two studies subsequently
2979 showed that aspartame administration (up to 4000 mg/kg bw/day for up to 90 days) had small effects
2980 on hepatic Phase 1 and Phase 2 metabolism in rodents after 45 days treatment but not after 90 days
2981 treatment (Tutelyan *et al.*, 1990). In a more recent study (Vences-Mejia *et al.*, 2006), male Wistar rats
2982 were dosed with aspartame (75 or 125 mg/kg bw/day) daily for 30 days. Using hepatic microsomal
2983 samples from liver, cerebrum (pooled) and cerebellum (pooled), the authors investigated CYPs by
2984 immunoblotting (CYPs 1A1, 1A2, 2B, 3A2 and 2E1) and enzymatic activity measurement
2985 (ethoxyresorufin O-deethylase, methoxyresorufin O-demethylase, pentoxyresorufin O-depentylase,
2986 benzyloxyresorufin O-debenzylase, 4-nitrophenol hydroxylase and erythromycin-N-demethylase). No
2987 effect of aspartame treatment was observed in the liver samples. However, bands corresponding to
2988 CYPs 1A1, 1A2, 2B, 3A2 were detectable in the cerebrum and cerebellum samples brain samples after
2989 aspartame treatment (but not in the control samples) and increases in all the enzyme activities were
2990 reported. The Panel noted that the findings have minimal relevance for human risk assessment.

2991 **3.2.6.3. Miscellaneous studies of aspartame**

2992 Alleva *et al.*, (2011) evaluated the ability of aspartame to induce angiogenesis in an *in vitro* model
2993 which used human endothelial cells (HUVEC) co-cultured in a matrix of human fibroblasts (IMR-90)
2994 following treatment with aspartame at concentrations of 20-100 µM in culture medium. The formation
2995 of blood vessels was associated with a transient release of the inflammatory cytokine IL-6 and the
2996 growth factor VEGF-A into the medium. Transient increases in the inflammatory cytokine IL-6 and
2997 the growth factor VEGF-A were also observed in the HUVEC cells following treatment with
2998 aspartame. This increase coincided with a temporary induction of ROS in HUVEC cells but not in
2999 IMR-90 fibroblasts thus concluding that generation of ROS is related to the target. The Panel noted
3000 that production of ROS could not be attributed to specific cell types but rather a general phenomenon.
3001 Furthermore, the authors did not evaluate the fate of aspartame in culture medium ascertaining
3002 whether it was hydrolysed to its usual metabolites or remained intact. For induction of angiogenesis,
3003 no positive and negative controls were reported. The Panel also noted that no formation of ROS in
3004 different *in vivo* and *in vitro* genotoxicity studies following treatment with aspartame or methanol
3005 (McCallum *et al.*, 2011a,b) has been observed. This suggests that the finding reported might be
3006 ascribed specifically to the conditions of the study. For these reasons, the Panel considered these data
3007 not relevant for the risk assessment of aspartame.

3008 Aspartame was administered to zebrafish (70 per dose group) in the diet (50 g/kg diet) for 14 days
3009 (Kim *et al.*, 2011). No effect of aspartame on survival was observed.

3010 Following a public call for data, preliminary investigations on a wide range of potential
3011 pharmacological and endocrine effects of aspartame, summarised in two data compendia (E1, 1972;
3012 E19, year not provided), were received. The compendia did not report any adverse effects.

3013 **3.2.7. Human studies of aspartame**

3014 **3.2.7.1. Epidemiological studies**

3015 The epidemiological data on aspartame were previously reviewed by SCF (2002). The Panel
3016 considered and agreed with the conclusions of SCF that there was no evidence for adverse effects of
3017 aspartame in the human population. The new epidemiology studies, published since the SCF opinion,
3018 are described below.

3019 **3.2.7.1.1. Recent epidemiological studies on artificially sweetened drinks and pre-term delivery**

3020 A large prospective cohort study (Halldorsson *et al.*, 2010) based on data from the Danish National
3021 Birth Cohort investigated the association between consumption of artificially sweetened or sugar
3022 sweetened soft drinks during pregnancy and subsequent pre-term delivery. Recruitment of the cohort
3023 of participants occurred between January 1996 and October 2002.

3024 The reasons cited by the authors for undertaking the analysis were:

3025 a) Both artificially and sugar-sweetened soft drinks might be associated with hypertension which is a
3026 known risk factor for preterm delivery.

3027 b) Intake of sugar-sweetened soft drinks has been related to the metabolic syndrome and type 2
3028 diabetes mellitus. High blood glucose concentrations have been linked to a shorter duration of
3029 gestation.

3030 c) Methanol is a metabolite of aspartame. Low dose methanol exposure has been linked to a shorter
3031 duration of gestation.

3032 The study population included 91,827 pregnant women from all over Denmark, equating to
3033 approximately 35% of all deliveries in Denmark during the study period. Of these, 59,334 (64.6%)
3034 women were analysed in this study, the main reason for attrition being failure to provide the required
3035 dietary information during pregnancy. Since the events leading to exclusion occurred before the
3036 outcome of pregnancy was known, they would not be expected to have given rise to bias. Analysis
3037 was restricted to each woman's first pregnancy registered during the study period. This restriction
3038 appears appropriate, since multiple pregnancies are a significant factor in preterm birth. It would not
3039 be expected to produce bias.

3040 Dietary exposures were obtained at about week 25 of gestation by a food frequency questionnaire
3041 (FFQ) covering the past month. Among other things, questions were asked about frequency of
3042 consumption (in servings) of each of: sugar-sweetened carbonated drinks; sugar-sweetened
3043 uncarbonated drinks; sugar-free carbonated drinks; and sugar-free uncarbonated drinks. It appears that
3044 no attempt was made to analyse exposures to other more important dietary sources of methanol such
3045 as fruit and vegetables. A subsample of 103 women completed a repeat FFQ at 33-35 weeks gestation.
3046 Spearman's correlation coefficients for the soft drink exposure variables in paired questionnaires were
3047 about 0.7. Due to the prospective design, any misclassification of exposures would be expected to bias
3048 risk estimates towards the null rather than to produce spurious associations between exposure and
3049 health outcome. It should be noted that the exposure assessment was for sweeteners in general and not
3050 specific for aspartame. Furthermore, the exposure assessment did not consider other dietary sources of
3051 sweeteners (e.g. sweeteners added to coffee, tea or other food products).

3052 Pre-term delivery was defined as delivery occurring before week 37 of gestation. Data on date of
3053 delivery and type of delivery (spontaneous or induced) were obtained from the Medical Birth Registry.
3054 Data on expected date of delivery were obtained from mothers at approximately week 12 of gestation
3055 (43% of pregnancies) or week 30 of gestation (56%), or from the Medical Birth Registry (1%).

3056 Statistical analysis was by logistic regression and apparently did not adjust for year of delivery. This
3057 could have produced bias if both consumption of soft drinks and thresholds for induction of delivery
3058 pre-term changed over the course of the study period. However, as the study period was only seven
3059 years, it is highly unlikely that any such bias would have been large. Analyses adjusted for seven non-
3060 dietary factors that were known to predispose to pre-term delivery – maternal age, maternal height,
3061 pre-pregnancy BMI, cohabitant status, parity, smoking during pregnancy, and familial socioeconomic
3062 status. This information was ascertained in pre-natal telephone interviews conducted at around weeks
3063 12 and 30 of gestation.

3064 Statistically significant trends were found in the risk of pre-term delivery with increasing consumption
3065 of artificially sweetened drinks (both carbonated and non-carbonated), but not for sugar-sweetened

3066 drinks. In the highest exposure groups (≥ 4 servings/day) the odds ratios relative to no consumption
3067 were 1.78 (95%CI 1.19-2.66) and 1.29 (95%CI 1.05-1.59) respectively for carbonated and non-
3068 carbonated artificially sweetened drinks. The associations with different categories of soft drinks were
3069 not mutually confounded, and the associations with consumption of artificially sweetened carbonated
3070 drinks did not differ demonstrably according to whether delivery was very early (<32 weeks) or only
3071 moderately or late pre-term.

3072 A notable finding was that the trend in risk for artificially sweetened carbonated drinks was strongest
3073 for medically induced pre-term deliveries, and was not clearly apparent for spontaneous pre-term
3074 deliveries. No analysis was made by reason for pre-term delivery (data on which may not have been
3075 available).

3076 The Panel noted that the prospective design and large size of the study sample were major strengths,
3077 and there were no major flaws in the methods used. However, risk estimates may have been inflated
3078 by residual confounding (including by year of delivery). In addition, the Panel noted that it was not
3079 possible to identify a mode of action for the association between pre-term delivery and an exposure
3080 measure based on consumption of artificially sweetened drinks. Not all confounding factors are
3081 accounted for (i.e. not taking into account other dietary sources of methanol, or distinguishing the use
3082 of a specific artificial sweetener, e.g. aspartame). Therefore, given these limitations, the Panel agreed
3083 with the authors who concluded that replication of their findings in another experimental setting is
3084 warranted.

3085 To explore whether the findings of Halldorsson *et al.* (2010) could be replicated, another study
3086 (Englund-Ögge *et al.*, 2012) investigated the relation between consumption of artificially sweetened
3087 and sugar-sweetened soft drinks during the first 4-5 months of pregnancy and subsequent pre-term
3088 delivery in a large cohort of Norwegian women.

3089 The authors used data from a prospective cohort of 90,700 pregnant women recruited during 1999-
3090 2008 (comprising 38.5% of those invited to participate) and analysed the data retrospectively. After
3091 exclusions for various specified reasons (e.g. not a singleton pregnancy, incomplete data on covariates,
3092 baby not born alive between gestational weeks 22 and 41), analysis was based on 60 761 women. The
3093 exclusions are unlikely to have been an important source of bias. In particular, where data were
3094 missing on covariates (6 630 women), this occurred before the outcome of pregnancy was known.

3095 Dietary exposures were ascertained at about week 22 of gestation by a semi-quantitative food
3096 frequency questionnaire covering the first 4-5 months of pregnancy. Among other things, six questions
3097 were asked about frequency of consumption (in 250 ml servings) of artificially and sugar-sweetened
3098 carbonated drinks (carbonated cola and other carbonated drinks) and non-carbonated drinks. From this
3099 information, total intakes of artificially sweetened and sugar-sweetened soft drinks were calculated.

3100 Pre-term delivery was defined as occurring before week 37 of gestation. Gestational age was
3101 determined by ultrasonography at gestational weeks 17-18. This information, and that about pregnancy
3102 outcome, was obtained from a national birth registry.

3103 Statistical analysis was by logistic regression. Analyses were carried out with and without adjustment
3104 for eight co-variates known to be associated with pre-term delivery – maternal history of previous pre-
3105 term delivery, maternal age at delivery, maternal pre-pregnancy BMI (calculated from self-report of
3106 weight and height), marital status, parity, smoking during pregnancy, maternal education, and total
3107 energy intake during the first half of pregnancy. The result of adjusting for co-variates was generally a
3108 reduction of the risk estimates. Some analyses also adjusted for consumption of the other type of
3109 beverage (sugar-sweetened or artificially sweetened), this increased risk estimates slightly.

3110 No significant trends were found in the risk of pre-term delivery with increasing consumption either of
3111 artificially sweetened drinks or of sugar-sweetened drinks. Small elevations of risk were observed with
3112 higher consumption of artificially sweetened soft drinks, but after adjustment for covariates, these

3113 reached statistical significance only when categories of consumption were aggregated to four levels,
3114 and then the odds ratio for the highest category (≥ 1 serving/day) was only 1.11 (95% CI 1.00-1.24) in
3115 comparison with never consumption. This was driven by an increase in spontaneous but not medically
3116 induced pre-term delivery. Associations with sugar-sweetened soft drinks tended to be somewhat
3117 stronger, with an adjusted odds ratio of 1.25 (95% CI 1.08-1.45) for consumption of at least one
3118 serving per day.

3119 This pattern of results contrasts with that reported by Halldorsson *et al.* The association of pre-term
3120 delivery with artificially sweetened soft drinks was much weaker and barely discernible, applied more
3121 to spontaneous than medically induced deliveries, and was exceeded by an association with
3122 consumption of sugar-sweetened soft drinks. The Panel noted that effects may have been
3123 underestimated because of non-differential inaccuracies in the assessment of dietary exposures, but the
3124 method was similar to that used by Halldorsson *et al.*, and the same for sugar-sweetened as for
3125 artificially sweetened soft drinks.

3126 In summary, both studies appear to have been well designed and conducted. Noting this, the Panel
3127 concluded that even at high level of exposure to artificially sweetened soft drinks the risk of pre-term
3128 delivery is likely to be small, if any. This observation could be a consequence of uncontrolled residual
3129 confounding, and the inconsistencies in the patterns of association reinforce this uncertainty. When
3130 findings from the two studies are considered together, they do not point to a hazard concerning the
3131 intake of artificially sweetened soft drinks.

3132 **3.2.7.1.2. Epidemiological studies of aspartame and cancer**

3133 In a Swedish case-control study, 209 patients with brain tumour were compared with 425 controls,
3134 who were selected from the Swedish Population Register (Hardell *et al.*, 2001) and matched to the
3135 cases for sex, age and region of residence. The focus of the study was exposure to ionising radiation
3136 and cellular telephones, but information was also collected about consumption of low-calorie drinks,
3137 most of which contained aspartame. Non-significant elevations of risk were observed for consumption
3138 of such drinks in relation to brain tumours overall (OR 1.24, 95%CI 0.72-2.14) and malignant brain
3139 tumours specifically (OR 1.70, 95% CI 0.84-3.44).

3140 The study had a high response rate, but was limited by its relatively small size, the basic assessment of
3141 exposure (low-calorie drinks were the only source of aspartame investigated in this study), and the
3142 potential for recall bias (because cases knew that they had a brain tumour) all of which could have led
3143 to spurious inflation of risk estimates.

3144 A US case-control study included 315 children with medulloblastoma/primitive neuroectodermal
3145 tumour diagnosed before the age of 6 years, and 315 control children (selected from the general
3146 population by random digit dialling) (Bunin *et al.*, 2005). Their mothers were interviewed
3147 retrospectively (on the telephone by trained interviewers) about their diet before (to ascertain the
3148 periconception period) and during pregnancy (second trimester). This was assessed by a modified food
3149 frequency questionnaire covering 112 items.

3150 In an unadjusted analysis, a significant trend of increasing risk was observed with more frequent
3151 consumption of low-calorie carbonated drinks (diet soda) in the pre-conception period. However, this
3152 was attenuated after adjustment for potential confounders, with an adjusted odds ratio of 1.3 (95% CI
3153 0.7-2.5 for ≥ 2 /day versus < 1 /month). There were no significant associations with reported frequency
3154 of consuming diet soda during midpregnancy.

3155 Response rates were reasonable, but the assessment of exposure to diet soda required recall after an
3156 interval of several years and therefore may not have been reliable. Furthermore, it served only as a
3157 proxy for exposure to aspartame (which at the time was the most widely used artificial sweetener in
3158 soft drinks), and other possible sources of aspartame were not evaluated.

3159 The authors concluded that their results generally did not support an association with aspartame, but
3160 the above limitations, and also the low statistical power, restrict the conclusions that can be drawn
3161 from this study.

3162 A paper by Gallus *et al.*, 2006 reports a linked set of case-control studies carried out in Italy between
3163 1991 and 2004 to assess the association of artificial sweeteners with nine types of cancer. Patients with
3164 incident, histologically confirmed cancers of the oral cavity and pharynx (598), oesophagus (304),
3165 colon (1225), rectum (728), larynx (460), breast (2569), ovary (1031), prostate (1294) and kidney
3166 (767) were compared with 7028 controls admitted to the same hospitals for acute, non-neoplastic
3167 disorders. Odds ratios for consumption of sweeteners other than saccharin (mainly aspartame) ranged
3168 from 0.71 to 1.62, and their upper 95% confidence limits from 1.00 to 3.14. The highest lower 95%
3169 confidence limit was 0.86 (prostate cancer).

3170 Dietary exposures were ascertained in an interview in hospital, which included a food frequency
3171 questionnaire with 78 items. Assessment of dietary exposure was restricted to usual diet in only the
3172 past two years, and did not cover all possible sources of artificial sweeteners – only sachets or tablets.
3173 However, the authors state that dietetic soft drinks had only recently been introduced in Italy, and that
3174 they were therefore unlikely to have contributed to the cancers in the age groups investigated.

3175 It seems likely that any misclassification of exposures will have been non-differential (i.e. similar for
3176 cases and controls), in which case the effect will have been to bias risk estimates towards the null.
3177 Thus, the results do not suggest a hazard for the cancers studied.

3178 A prospective cohort study by Lim *et al.* (2006) was based on data from the NIH-AARP Diet and
3179 Health Study. The analysis included 285,079 men and 188,905 women aged 50 to 71 years at entry to
3180 the cohort, which was drawn from members of the American Association of Retired Persons (AARP)
3181 in 8 study areas in the US. In this cohort, risk of haematopoietic cancer (1888 cases) and malignant
3182 glioma (315 cases) during five years of follow-up (1995-2000) was examined in relation to daily
3183 intake of aspartame assessed at baseline. For haematopoietic cancer, intake of ≥ 600 mg aspartame per
3184 day carried no elevation of risk (RR 0.98, 95% CI 0.76-1.27), and there was no association with
3185 glioma and consumption of aspartame (RR 0.73, 95% CI 0.46-1.10). Assessment of exposure to
3186 aspartame was through a FFQ, and covered aspartame-containing drinks as well as addition of
3187 aspartame to coffee and tea. The authors concluded that their prospective study suggested that
3188 aspartame consumption derived from its main source, aspartame-containing beverages does not raise
3189 the risk of haematopoietic or brain malignancies.

3190 The Panel noted that the major strengths of this investigation were its prospective longitudinal design,
3191 the high number of participants, exposures being assessed at baseline and therefore unbiased by
3192 knowledge of disease outcome. Ascertainment of cancers was reliable. Confounding is unlikely to
3193 have been a major problem, although there was no adjustment for socio-economic status (which has
3194 shown some relation with brain cancer).

3195 In a small pilot case-control study of brain cancer conducted in southern France, 122 incident adult
3196 cases were compared to 122 controls with other neurological diagnoses (Cabaniols *et al.*, 2011). There
3197 was no association with aspartame consumption during the past five years of at least once per week
3198 (OR 1.02, 95% CI 0.57-1.85). However, information on the method of dietary assessment is limited,
3199 and there was no attempt to control for potential confounding factors other than sex and age. In view
3200 of this, and the low statistical power of the study (reflected in the confidence interval), the non-
3201 positive finding provides little reassurance of an absence of hazard.

3202 The risk of lymphatic and haematopoietic cancers in relation to consumption of diet soda and
3203 aspartame sweeteners added at the table was examined in two US cohorts – one of 77,218 female
3204 registered nurses and one of 47,810 male health professionals – followed prospectively from 1984 and
3205 1986 to 2006 (Schernhammer *et al.*, 2012).

3206 Exposure to aspartame through consumption of diet soda and packets used at table was assessed by
3207 semi-quantitative food-frequency questionnaires repeated at four-yearly intervals, and at each point in
3208 follow-up was characterised by the average from all questionnaires that the participant had completed
3209 up to that time. Various potential confounding factors (e.g. weight, smoking habits, physical activity)
3210 were also assessed through repeated questionnaires. Cancer outcomes were ascertained from biennial
3211 questionnaires, state tumour registries and death records, and where possible, diagnoses were verified
3212 using medical records. Analysis was by Cox regression.

3213 During follow-up, 1,324 subjects developed non-Hodgkin lymphoma (NHL), 285 multiple myeloma,
3214 and 339 leukaemia (mostly myeloid leukaemia). After adjustment for potential confounders, the
3215 category of highest aspartame intake (≥ 143 mg/day, as defined by the authors) was associated with a
3216 significantly elevated relative risk of NHL (1.64, 95%CI 1.17-2.29) and of multiple myeloma (3.36,
3217 95%CI 1.38-8.19) in men. However, there was no consistent trend in risk with increasing exposure,
3218 and there were no corresponding elevations in risk in women. No clear association with leukaemia was
3219 apparent in either men or women. The authors speculated that the differential findings for men and
3220 women might reflect differences in the activity of alcohol dehydrogenase type 1, which converts
3221 methanol (a metabolite of aspartame) to formaldehyde.

3222 Major strengths of this study are its prospective design, the substantial number of cancer cases, the
3223 repeated assessment of dietary intake and the fact that various potential confounders were addressed.
3224 However, the positive findings can be given little weight, given their limitation to men, the small
3225 relative risks observed, and the lack of clear dose-response relationships. The authors' proposed
3226 explanation for the differential associations in men and women is unconvincing in the absence of data
3227 on other more important dietary sources of methanol.

3228 Overall, the Panel considered that the results of these epidemiological studies do not suggest an
3229 increased risk associated with aspartame consumption for the types of cancer examined.

3230 **3.2.7.2. Single dose studies in humans**

3231 A number of single dose studies on the kinetics of aspartame in human subjects have been carried out;
3232 these are described in Section 3.1.

3233 In one study, single loading doses of aspartame (34 mg/kg bw) and, two weeks later, of L-
3234 phenylalanine (19 mg/kg bw) were administered in orange juice to two female adolescents (E66,
3235 1973). The authors of the study reported that the two subjects tolerated the doses well. In another
3236 study (E110, 1979), six subjects who claimed to be sensitive to monosodium glutamate (Chinese
3237 Restaurant Syndrome) were given a loading dose of 34 mg aspartame/kg bw or 1000 mg sucrose/kg
3238 bw in a cross-over study. The test doses were dissolved in orange juice. Although one subject reported
3239 slight nausea after aspartame, no symptoms of Chinese Restaurant Syndrome were reported.

3240 **3.2.7.3. Repeat dose studies in humans**

3241 A number of repeat dose studies on the kinetics of aspartame in human subjects have been carried out,
3242 and some of them are described in Section 3.1.

3243 Several repeat dose studies in healthy non-obese human subjects have been performed with aspartame.
3244 In preliminary studies (E23, 1972) 31 men and 38 non-pregnant women (age 21-45 years) were
3245 administered aspartame under double blind conditions in capsules containing placebo, 200 mg
3246 aspartame or 300 mg aspartame. The subjects took an appropriate number of capsules per day to
3247 deliver increasing doses of aspartame (or placebo) over a six-week period (600, 1200, 2400, 4500,
3248 6300 and 8100 mg/day in a weekly dose escalation protocol) followed by a one week with no
3249 treatment. Standard clinical chemistry profiles were analysed together with methanol, determined by
3250 gas chromatography in serum and urine at weeks 4 and 6. All 69 subjects completed the study, and
3251 there were no obvious physical or biochemical differences between the aspartame and placebo groups
3252 before, during or at the end of the study. No significant differences in glucose and insulin homeostasis

3253 were identified at weeks 0 or 6, or during follow up (week 7). There was no accumulation of
3254 phenylalanine or tyrosine in subjects taking aspartame; phenylalanine was not detected in urine in
3255 weeks 0, 3, 6 or 7. The only 'adverse' symptoms reported were mild and often contradictory (loose
3256 stools/constipation, increased/decreased appetite and headache).

3257 A similar short term (6-week) study was conducted in obese adults (ages 21-70 years; weight
3258 exceeding more than 20% of the mean normal weight for height, sex, frame and age) (E24, 1972). Of
3259 the 95 subjects entering the study, 84 successfully completed the 6 weeks of treatment (44 on
3260 aspartame and 40 on placebo). The dose regime was the same as that described in E23 (1972), as were
3261 the biochemical analyses. As with the normal adults, no obvious physical or biochemical differences
3262 between the aspartame and placebo group before, during or at the end of the study were reported.
3263 According to the authors, few complaints were reported during the study and none were serious (e.g.
3264 loose stools, constipation, decreased appetite, increased appetite, headache). The incidence of these
3265 complaints was slightly greater among females than males, and among the aspartame users than
3266 placebo subjects. Subjects in both groups lost a significant amount of weight between the initial
3267 weighing and the end of the study, but the difference in weight loss between two groups was not
3268 significant.

3269 A follow up study (E60, 1973) was conducted in subjects who agreed to continue from the study
3270 described above (E23, 1972) together with a number of new recruits. This follow up study ran for 21
3271 weeks, so continuing subjects received aspartame for a total of 27 weeks and the new recruits were
3272 dosed for 21 weeks. Subjects from the previous study stayed on the same preparation (either aspartame
3273 or placebo) as they had received during the short-term study, but all the new recruits received
3274 aspartame. All subjects were dosed at 1800 mg aspartame/day (2 x 300 mg capsules, 3 x daily).
3275 Laboratory tests conducted during weeks 6, 12, 20 and 21 revealed some variability in results, but this
3276 was not associated with consumption of aspartame. There were no consistent differences in glucose
3277 and insulin homeostasis between aspartame and placebo groups and, despite fluctuations in plasma
3278 phenylalanine and tyrosine levels, there was no accumulation of either amino acid in male or female
3279 subjects. No phenylpyruvic acid was detected in urine. No changes in weight and no product-related
3280 side effects were reported; the symptoms that were reported were of a similar nature to those in the
3281 previous study.

3282 Aspartame tolerance was also evaluated in a 13-week double-blinded study in children and
3283 adolescents, aged 2-20 years (E61, 1972). Five groups of children (ages 2-3, 4-6, 7-9, 10-12 and 13-20
3284 years) were randomly assigned to receive sucrose or aspartame at doses of 39.5 – 58.1 mg/kg/day, and
3285 older subjects (ages 13-20 years) also took aspartame capsules (200 mg, 3 x daily). Subjects returned
3286 for evaluation at the end of weeks 1, 3, 5, 7, 9, 11 and 13. Clinical chemistry profiles indicated some
3287 minor differences between aspartame and placebo groups, but all were considered clinically trivial and
3288 none persisted. No compound-related differences in profiles were detected at the end of the study.
3289 Both groups exhibited a slight decline in plasma phenylalanine levels during the study but tyrosine
3290 levels remained unchanged and there were no trends in phenylalanine/tyrosine ratios. Urinary
3291 phenylpyruvic acid measurements were negative and both serum and urinary methanol levels (in 33
3292 subjects selected at random) were below the limit of detection at all times. There was slight weight
3293 gain during the study in all groups, consistent with the age of the participants. No severe adverse
3294 effects were reported and the minor complaints received were similar for aspartame and placebo
3295 groups. Hives and rashes were observed in four individuals but these were not related to aspartame
3296 consumption. No eye abnormalities or changes in acne incidence or severity were observed.

3297 A metabolic study was designed to evaluate the effect of added monosodium glutamate (MSG) plus
3298 aspartame upon plasma amino acid levels (E95, 1977). Three male and three female subjects were
3299 given, in a cross-over manner, a test meal or the same test meal containing aspartame at 34 mg/kg and
3300 MSG at 34 mg/kg. Blood samples were collected up to 8 hours. The addition of aspartame (34 mg/kg)
3301 to the meal increased the phenylalanine content from 43 to 64 mg/kg. The addition of aspartame plus
3302 MSG had no effects upon the plasma glutamate curves, the plasma amino acids levels, and the plasma
3303 aspartate levels. The plasma levels of phenylalanine and tyrosine after ingestion of MSG and

3304 aspartame were only slightly higher than those noted after the ingestion of the test meal alone, and all
3305 levels were within the normal postprandial limits.

3306 Leon *et al.* (1989) conducted a study of the effects of aspartame involving 101 subjects (ages 18-62
3307 years). This was a randomised, double blind, placebo-controlled, parallel-group design. Participants
3308 included 57 women and 51 men, randomly assigned to either the aspartame ($n = 53$) or placebo ($n =$
3309 55) groups. Exclusion criteria were well-defined and included body weight greater than 20% above
3310 normal range. Subjects consumed three capsules per day containing either 300 mg aspartame or 300
3311 mg microcrystalline cellulose plus 0.9 mg silicon dioxide for 24 weeks. Compliance was verified by
3312 counting capsules and subjects were asked to avoid additional sources of aspartame. The approximate
3313 dose of aspartame was 75 mg/kg bw/day. No treatment-related haematological changes, consistent
3314 alterations in clinical chemistry and analyses or urinary abnormalities were observed and no
3315 significant differences in vital signs or body weight were noted. In addition, no differences were
3316 observed in relation to blood formate or methanol; urinary Ca^{2+} or formate; serum folate; or serum
3317 lipids. There were no significant changes in amino acid profiles and no evidence of accumulation of
3318 phenylalanine or tyrosine. Various adverse events were reported which were all considered by the
3319 authors as frequently occurring minor ailments and mild in nature.

3320 In a controlled diet study (Porikos and Van Italie, 1983) in which 21 men (aged 24-45; 15 obese, 6
3321 non-obese) were given a baseline diet (25-30% of calories from sucrose) alternating with a calorie-
3322 restricted diet (25-30 % of calories from sucrose (days 1-6 and 19-30, sweetness being replaced with
3323 aspartame on days 7-18, so volunteers ate significantly less calories on days of aspartame
3324 consumption) for 12 days at a time, the ALT and AST levels in serum were elevated (but remained
3325 within the normal range) on the baseline diet, fell on the aspartame-containing diet then rose again
3326 when the baseline diet was reintroduced. There were also small increases in blood urea nitrogen
3327 (BUN) levels in subjects on the aspartame containing diet, but renal function remained within normal
3328 parameters. Serum triglycerides decreased by 33% on the aspartame diet (from 130 mg/dl to 87
3329 mg/dl). The Panel noted that the dose of aspartame was not specified and that there were other changes
3330 in the diet, including changes in its caloric content.

3331 A short-term tolerance study was conducted in adult PKU heterozygotes (parents of homozygote
3332 children) in three different laboratories (E25, 1972). A total of 65 subjects (men and women between
3333 the ages of 21 and 45) were administered under double blind conditions capsules containing placebo,
3334 200 mg aspartame or 300 mg aspartame. The subjects took an appropriate number of capsules per day
3335 to deliver increasing doses of aspartame (or placebo) over a six week period (600, 1200, 2400, 4500,
3336 6300 and 8100 mg/day in a weekly dose escalation protocol) followed by a one week with no
3337 treatment. Total daily phenylalanine from aspartame was 340, 670, 1350, 2530, 3540, 4550 mg/day,
3338 respectively. All 65 subjects completed the study and there were no biochemical differences between
3339 the aspartame and placebo group either initially or at the end of the study in two out of the three
3340 groups. However, in one of the laboratories, a significantly higher mean initial value on BUN was
3341 reported in the placebo group, which was not detected at the end of the study. This laboratory reported
3342 also a significantly higher mean value in haematocrit in the aspartame group at the end of the study;
3343 this value was however within the normal range. No significant differences in glucose and insulin
3344 levels were identified at weeks 0 or 6, or during follow up (week 7). There were no changes in serum
3345 phenylalanine, tyrosine levels (mean levels were below the 8 mg set as upper limit for women) or in
3346 the ratio phenylalanine/tyrosine in subjects taking aspartame after fasting overnight and without
3347 preceding aspartame intake for more than 12 hours. No abnormal findings were reported on the
3348 urinary phenylalanine metabolites. The only complaints reported were a feeling of fullness and some
3349 unusual bowel movements.

3350 Long-term tolerance studies were also conducted in adult PKU heterozygotes. In a double-blind study
3351 a total of 52 subjects (men and women between the ages of 21 and 45) were randomly assigned to be
3352 dosed at 1.8 g aspartame/day (2 x 300 mg capsules, 3 x daily) or placebo for 21 weeks (E67, 1973).
3353 Laboratory tests (complete blood count, urinalysis, partial thromboplastin, prothrombin time, BUN,
3354 thyroxine, bilirubin, SGOT, alkaline phosphatase, uric acid, creatinine, cholesterol, triglycerides)

3355 conducted during weeks 6, 12, 16, 20 and 21 revealed no differences between the groups in the final
3356 laboratory values as well as in the final serum glucose and insulin levels. The only significant
3357 difference between the treatment groups occurred in the phenylalanine levels at week 16. No abnormal
3358 findings on the phenylalanine metabolism were observed in any subject.

3359 In another study, six healthy adult subjects (3 males and 3 females) and five females clinically
3360 identified by the authors as PKU heterozygous subjects were administered aspartame at 100 mg/kg bw
3361 dissolved in orange juice (E109, 1978). The PKU heterozygous subjects were selected after detection
3362 of a significant difference in plasma phenylalanine levels following loading with phenylalanine.
3363 Plasma and erythrocyte samples were collected up to 8 hours after the load. Plasma aspartate levels
3364 were not significantly affected by aspartame administration in either normal subjects or presumed
3365 PKU heterozygous subjects. Levels for glutamate, asparagine and glutamine were also unchanged
3366 after aspartame loading in the two groups. Plasma phenylalanine levels increased significantly after
3367 aspartame loading in both normal subjects and PKU heterozygous subjects. The plasma phenylalanine
3368 levels in PKU heterozygous subjects ingesting aspartame at 100 mg/kg bw were similar to those
3369 observed in normal subjects ingesting aspartame at 200 mg/kg bw. In the normal subjects plasma
3370 phenylalanine levels increased from fasting levels of $5.6 \pm 1.2 \mu\text{M}$ to values in the normal postprandial
3371 range of $11.1 \pm 2.49 \mu\text{M}$ and returned to baseline by 8 hours. In heterozygous subjects, the
3372 postprandial levels were higher; $16.0 \pm 2.25 \mu\text{M}$. Plasma tyrosine levels increased after aspartame
3373 loading in both groups, with higher levels in normal subjects. Levels of aspartate in erythrocytes were
3374 unchanged after aspartame administration in both groups, whereas phenylalanine and tyrosine levels in
3375 erythrocytes increased significantly over baseline.

3376 Another study (E26, 1972) assessed the tolerance of loading doses of aspartame by PKU homozygous
3377 children. Two 14-year-old PKU homozygous boys were given a loading dose of 34 mg/kg bw
3378 aspartame in orange juice and 2 weeks later they were given a molecular equivalent amount of
3379 phenylalanine (19 mg/kg bw) in orange juice. One of the boys was on a liberalized Lofenalac diet
3380 (infant powder formula to replace milk in the diet of PKU sufferers) with an allowable phenylalanine
3381 dietary intake of 70 mg/kg/day; the other boy was on a well-controlled Lofenalac diet (phenylalanine
3382 intake of 17 mg/kg/day). The subject on a liberalised diet received a total amount of 3228 mg of
3383 phenylalanine (total dietary intake/occasion: 2539 mg + phenylalanine added from loading dose: 689
3384 mg), which was well within his permitted phenylalanine range during the loading study. The subject
3385 on a restricted diet received a total amount of 2037 mg of phenylalanine (total dietary intake/occasion:
3386 965 mg + phenylalanine added from loading dose: 1072), which far exceeded his dietary limitation.
3387 The patient on a liberalised diet was excreting phenylalanine metabolites in large quantities, whereas
3388 the patient on a restricted diet did not excrete phenylpyruvic acid after either loading. The serum
3389 analysis indicated that the loading dosages of aspartame and phenylalanine did not cause any
3390 significant increase in either phenylalanine or tyrosine levels.

3391 Overall, the Panel concluded that no significant adverse effects were observed following repeated
3392 administration of aspartame for different durations and at different dose levels of aspartame either in
3393 healthy (adults, adolescents and infants) or in PKU heterozygous subjects.

3394 **3.2.7.4. Effect of Aspartame on behaviour and cognition**

3395 *Children*

3396 Kruesi *et al.* (1987) evaluated the effect of sugar and aspartame on 'aggression and activity' in
3397 preschool boys (ages 2 to 6 years) who were identified as 'sensitive to sugar' by their parents. The
3398 study was a double-blind cross-over challenge with aspartame (30 mg/kg bw), sucrose (1.75 g/kg bw),
3399 saccharin (amount not specified) and glucose (1.75 g/kg bw) to sugar-responsive (children described
3400 as being sensitive to sugar; $n = 14$) and age-matched control boys ($n = 10$). The sweeteners were given
3401 in a lemon-flavoured drink each sweetener was given on two occasions; once in a laboratory setting,
3402 and once 4 days later in a home setting. Children were scored for 'aggression and activity' by
3403 researchers during the laboratory playroom challenge, and by their parents in the days following the

3404 challenge to detect any delayed reaction, and during the home challenge. Washout periods of 5–7 days
3405 occurred between challenges. There was no significant difference in scores of aggression between the
3406 sugar-responsive and age-matched control boys following any of the four treatments. However, lower
3407 but not significantly different activity was scored during the aspartame challenges in both sugar-
3408 responsive and the age-matched control children (recorded by means of an actometer) but the
3409 differences were not apparent to the observers.

3410 In a double-blind randomised placebo controlled trial 25 preschool children and 23 school age children
3411 were provided with diets containing aspartame (32-38 mg/kg bw/day) or Sucrose (4500-5600 mg/kg
3412 bw/day) or saccharin (10-12 mg/kg bw/day) as placebo for 3 week periods. The study was well
3413 controlled with all food being provided to volunteers for the study period and measures of dietary
3414 compliance by means of biochemical tests. No adverse effects attributable to aspartame or dietary
3415 sucrose on children's behaviour or cognitive function were noted (Wolraich *et al.*, 1994).

3416 In a randomised, double blind, and placebo-controlled crossover study Shaywitz *et al.* (1994) assessed
3417 the effect of aspartame on behaviour and cognitive function of children with attention deficit disorder.
3418 The dose of aspartame was 34 mg/kg bw/day. The children ($n = 15$, 11 males, 4 females, ages 5 to 13
3419 years) were given capsules of either aspartame or placebo (microcrystalline cellulose) each morning
3420 for a 2-week period. Parents were instructed to provide an aspartame-free diet during the study. No
3421 effect was found on cognitive, attentive or behavioural testing or on urinary levels of neurotransmitters
3422 (noradrenaline, adrenaline, dopamine, homovanilic acid and 5-hydroxyindoleacetic acid), but plasma
3423 tyrosine and phenylalanine levels were higher 2 hours after the aspartame treatment. Plasma tyrosine
3424 level values were not provided. Plasma phenylalanine levels are only reported graphically, and
3425 increased from approximately 60 μM at baseline to approximately 85 μM two hours after aspartame
3426 dosing. The Panel noted that the changes in plasma phenylalanine levels were consistent with the
3427 toxicokinetic studies.

3428 The studies of Roshon and Hagen (1989) and Saravis *et al.* (1990) evaluating the effect of aspartame
3429 on the learning, behaviour and mood of children also report negative results. The Panel noted that
3430 there were no convincing effects of aspartame in children in these studies.

3431 *Adults*

3432 A double-blind randomised crossover trial (Lapierre *et al.*, 1990) with 10 healthy adult volunteers (6
3433 men, 4 women, ages 21–36 years) evaluated the effect of a single dose of aspartame (15 mg/kg bw) or
3434 placebo capsules on mood, cognitive function, and reaction time. No effect was observed on any of the
3435 parameters measured (i.e. hunger, headache, memory, reaction time, or cognition) during the study
3436 despite elevation of plasma phenylalanine levels following consumption (data values not reported).

3437 In another study (Ryan-Harshman *et al.*, 1987), healthy males age 20 to 35 years ($n = 13/\text{group}$) were
3438 given capsules containing placebo or aspartame (5 or 10 g; single dose) in a randomised crossover
3439 design. Plasma phenylalanine levels increased from a mean of 71 μM at baseline, by 55 and 193 μM at
3440 90 min after consumption of 5 and 10 g aspartame, respectively. Plasma tyrosine levels increased by
3441 25 and 47 μM , from a baseline of 77 μM at 90 min after consumption of 5 and 10 g aspartame,
3442 respectively. No behavioural effects (food consumption, mood or alertness) were observed.

3443 Pivonka and Grunewald (1990) compared the effect of water, and aspartame and sugar-containing
3444 beverages on mood in 120 young women and found no effect on self-reported surveys of mood.

3445 The acute and chronic studies by Stokes *et al.* (1991 and 1994), respectively, investigated the effect of
3446 aspartame ingestion on cognitive performance. The acute study (Stokes *et al.*, 1991) involved 12
3447 healthy certified pilots (four females and eight males). The study was double-blinded with each
3448 subject undertaking testing on 5 occasions, with at least 1 week between treatments that were given in
3449 random order among the 12 participants. Participants were tested for baseline values, then given
3450 placebo capsules, aspartame (50 mg/kg bw), or ethyl alcohol (positive control, estimated dose to raise

3451 plasma alcohol 0.1%), followed by a post-test with no treatment. For all treatments, participants
3452 consumed orange juice with either a trace or the test dose of alcohol, and capsules containing either
3453 placebo (dextrose) or aspartame, all participants consumed a small carbohydrate meal prior to
3454 treatments. Cognitive performance was tested using the SPARTANS cognitive test battery (a sensitive
3455 test to detect changes in performance of complex tasks required for aircraft operations). Cognitive
3456 impairment was detected in several tasks following consumption of the low dose of alcohol but not
3457 aspartame or placebo treatments.

3458 The follow up study (Stokes *et al.*, 1994) was undertaken in 12 subjects (college students, sex not
3459 reported) received placebo capsules or aspartame capsules (50 mg/kg bw/day) for 9 days, or an acute
3460 dose of ethyl alcohol to achieve 0.1% blood ethanol levels as described above. All participants
3461 received the placebo and ethanol treatments once and the aspartame treatment twice with a 7-day
3462 period between treatments. On the last day of treatment periods plasma phenylalanine levels averaged
3463 59 µM following placebo treatments and 121.5 µM following aspartame consumption. Forty-seven
3464 task variables were measured and significant differences between pre- and post-test results and
3465 aspartame treatment were detected for three tasks. However, an improvement, rather than impairment,
3466 of function was observed in participants following the aspartame treatments, which the authors
3467 described as unexpected and attributed to chance.

3468 In a three-way crossover double-blind study a group of 48 healthy volunteers (24 men, 24 women,
3469 ages 18–34 years) received treatments consisting of aspartame, sucrose and placebo administered for
3470 20 days (Spiers *et al.*, 1998). Twenty-four participants received a high dose of aspartame (45 mg/kg
3471 bw/day) and the remaining received a low dose of aspartame (15 mg/kg bw/day). Acute effects were
3472 evaluated on day 10 of each treatment, with testing starting 90 min after consumption of test material;
3473 chronic effects were evaluated on day 20. Plasma phenylalanine levels increased dose-dependently
3474 with aspartame consumption, but no cognitive, neurophysiologic and behavioural effects were
3475 observed.

3476 Adult patients (ages 24-60 years) undergoing treatment for depression were compared with non-
3477 depressed controls (n = 8) who believed themselves to be sensitive to aspartame (n=5). Subjects
3478 received aspartame (30 mg/kg bw/day) or placebo (sucrose) in capsules for a period of 7 days.
3479 Subjects monitored themselves and self-scored the severity of a list of symptoms, including headache,
3480 nervousness, dizziness, nausea, feeling 'blue' or depressed, temper outbursts. One patient suffered
3481 retinal detachment during the placebo phase; the researchers speculated that consumption of aspartame
3482 prior to placebo might have been responsible. Another patient experienced a conjunctival haemorrhage
3483 during the aspartame phase. These events lead to a halt of the study. Only 11 participants completed
3484 the study, but total points for adverse effects were compared for all 13 participants. Higher point
3485 values were recorded by depressed patients due to consumption of aspartame (mean = 40) compared to
3486 during consumption of placebo (mean = 10), but non-depressed participants recorded similar points
3487 during aspartame (mean = 13) and placebo (mean = 15) phases (Walton *et al.*, 1993). The Panel noted
3488 that attribution of ocular effects to aspartame irrespective of whether they occurred during placebo or
3489 aspartame treatment appeared inappropriate.

3490 The Panel noted the limited number of participants, the short duration and the inconsistency of the
3491 reporting of the results in all these human studies. Overall, the Panel concluded that the weight-of-
3492 evidence suggested that aspartame ingestion had no effect on behaviour or cognitive function.

3493 **3.2.7.5. Effects of aspartame on seizures**

3494 A double-blind study was undertaken in children recently diagnosed with generalised absence seizures
3495 or also called petit mal seizures to ascertain whether aspartame would exacerbate the occurrence of
3496 such seizures (Camfield *et al.*, 1992). After eating breakfast of their own choice, children (n = 10)
3497 drank orange juice sweetened with either aspartame (40 mg/kg bw) or sucrose (1 g sucrose for every
3498 25 mg aspartame) to achieve similar sweetness. For six hours following consumption of the juice the
3499 number and length of spike-wave bursts, indicative of an absence seizure, were determined using

3500 EEG40 recordings. Each child was tested once with each substance, on two consecutive days,
3501 treatments were assigned in a random fashion. No information was provided regarding whether lunch
3502 or snacks were given. There were no significant differences in either the frequency or duration of
3503 spike-wave bursts; however, when the two factors were combined, the total time spent in spike-wave
3504 per hour of observation was significantly higher in children after consumption of aspartame compared
3505 with sucrose (Camfield *et al.*, 1992). The Panel noted that combination of the two factors into a single
3506 measure was not adequately explained, and lack of control of food and drink intake before and after
3507 dosing may have affected the results.

3508 In a randomised double-blind placebo controlled, crossover study (Shaywitz *et al.*, 1994) 34 mg
3509 aspartame/kg bw was administered to epileptic children (n = 10) for two weeks to ascertain the
3510 induction of seizures. Measurements prior to and following treatments included seizure incidence,
3511 overall activity and behaviour, EEG recordings, adverse experiences, liver function, urine analysis,
3512 and plasma levels of amino acid, methanol, formate, glucose, and monoamines and metabolites.
3513 Children ate their normal diet, but were asked to exclude a list of foods containing aspartame. There
3514 was no difference in the occurrence of seizures during the aspartame or placebo treatments. Increased
3515 levels of phenylalanine and tyrosine during aspartame treatments were recorded.

3516 In another randomised double-blind placebo-controlled crossover study (Rowan *et al.*, 1995) induction
3517 of seizures following aspartame ingestion in self-reported aspartame-sensitive individuals (six adults
3518 and two children) was assessed. The subjects received placebo or 50 mg aspartame /kg bw in three
3519 doses throughout the day, on days 2 and 4. EEG recordings were performed for five consecutive days.
3520 Meals were standardised throughout treatment. No clinical seizures were observed in subjects during
3521 the study. Electrographic seizures were recorded in two subjects on days consuming the placebo. Sleep
3522 variables were also measured, but no effect of aspartame was observed.

3523 The Panel noted that there was no evidence of effects of aspartame on seizures.

3524 **3.2.7.6. Effect of aspartame on headaches**

3525 The possible effect of aspartame on headaches has been investigated in various studies, which reported
3526 conflicting results. Some reported no effect and others suggested that a small subset of the population
3527 may be susceptible to aspartame-induced headaches. The number of existing studies was small, and
3528 several had high participant dropout rates, making interpretation of results difficult.

3529 A double-blind crossover trial (Schiffman *et al.*, 1987) with 40 individuals (12 males, 28 females; ages
3530 19–69) who had previously reported suffering headaches when they consumed aspartame, was a well-
3531 controlled study with patients being housed and monitored in an inpatient unit. Participants were
3532 monitored for 2 days, and then challenged with capsules of aspartame (30 mg/kg bw) or placebo
3533 (microcrystalline cellulose) on days 3 and 5, with day 4 being a washout day. Diet and extraneous
3534 variables were controlled. There was no evidence of an effect of aspartame, as incidence of headache
3535 after consumption of aspartame (35%) or after the placebo (45%) was similar (Schiffman *et al.*, 1987).

3536 In contrast, the study by Koehler and Glaros (1988) had volunteers who suffered with migraines and
3537 stayed in their normal environments during a double-blind crossover study with three phases: a 4-week
3538 baseline phase and two four-week experimental phases, with a 1-week washout phase between
3539 treatments. Participants (two males, eight females; ages 18 to 47 years) consumed capsules of
3540 aspartame (300 mg) or placebo (microcrystalline cellulose) and self-recorded incidence of headaches
3541 and dietary information. The incidence of headaches did not differ from baseline during the placebo
3542 phase. Five of the eleven participants reported a higher number of migraines during the aspartame
3543 phase compared to during the baseline or placebo phases. The mean number of headaches reported
3544 was 1.72, 1.55, and 3.55 during the baseline, placebo, and aspartame phases, respectively. No
3545 differences were reported in the intensity or duration of migraine headaches. Dietary records did not
3546 show any substantial changes in diet among phases. The high dropout rate, from 25 to 11 participants
3547 in this study makes interpretation of the results difficult.

3548 Patients at a headache unit were asked to complete a survey reflecting whether they felt that alcohol,
3549 aspartame or carbohydrate intake were triggers of headaches (Lipton *et al.*, 1989). Of the 171 patients
3550 who completed the survey, 8.3% reported aspartame as a trigger of headaches, and often a migraine
3551 headache. As this was significantly higher than the response to carbohydrates (2.3%), the authors
3552 concluded that aspartame might be a migraine headache trigger for some individuals. The Panel
3553 considered ‘the power of suggestion’ of having aspartame listed as a possible trigger of headaches to
3554 be a major limitation of this study.

3555 Van den Eeden *et al.* (1994) conducted a double-blind randomised crossover trial with 32 subjects
3556 self-diagnosed as sensitive to aspartame. Only 18 participants completed the full protocol, as other
3557 subjects withdrew for various reasons including adverse effects. Subjects took capsules containing
3558 either aspartame or placebo (microcrystalline cellulose) three times a day to achieve a dose of 30
3559 mg/kg bw/day for seven days. A significantly higher ($p = 0.04$) occurrence of self-reported headaches
3560 was recorded following exposure to aspartame (33% of days) compared to placebo (24% of days). The
3561 subjects who had excess headaches following aspartame dosing were those who had, at the beginning
3562 of the study, indicated they were ‘very sure’ that they were susceptible to aspartame-induced
3563 headaches. In contrast, those subjects who classified themselves as ‘somewhat or not sure’ reported
3564 similar headache incidence during aspartame and placebo exposure periods. The authors conclude that
3565 these results indicated that a small subset of the population was susceptible to aspartame-induced
3566 headaches (Van den Eeden *et al.*, 1994). The Panel consider that with such a low number of
3567 participants it was not possible to draw a conclusion.

3568 The Panel noted that there was no convincing evidence of effects of aspartame on headache.

3569 **3.2.7.7. Effects of aspartame on Hunger/Satiation and Appetite**

3570 Drewnowski *et al.* (1994) fed 12 obese and 12 lean women one of four breakfast preloads sweetened
3571 with 50 g sucrose or 500 mg aspartame, or aspartame plus 50 g maltodextrin, in a cross-over design.
3572 As such all subjects were tested with all treatments. Subsequent food intake and calorie consumption
3573 during lunch, snack, and dinner was recorded and were reported not to be affected by the sweetener
3574 consumed in the preload (Drewnowski *et al.*, 1994).

3575 A meta-analysis of 16 randomised controlled trials assessing the effect of aspartame consumption on
3576 energy intake with observations on weight loss and weight maintenance was undertaken by de la
3577 Hunty *et al.* (2006). The studies that have addressed the question of the effect of aspartame on appetite
3578 and body weight, that have actually measured food consumption, have shown that aspartame does not
3579 increase caloric intake. Significant reduction of energy intake with consumption of aspartame was
3580 observed, except when the control was a non-sucrose control such as water (de la Hunty *et al.*, 2006).

3581 A number of studies have focused on the effects of aspartame on appetite/hunger and food intake
3582 (Appleton and Blundell, 2007; Reid *et al.*, 2007; Appleton *et al.*, 2004; Van Wymelbeke *et al.*, 2004).
3583 The reported results were inconsistent but some studies suggested that the experience of sweet taste
3584 without a calorific intake had an influence on appetite for both sweet and savoury foods. It should be
3585 noted that no studies focused specifically on the effects of aspartame on body weight.

3586 **3.2.7.8. Allergenicity of aspartame**

3587 Butchko *et al.* (2002) reviewed all published papers from 1980 onwards reporting allergic-type
3588 reactions attributed to aspartame exposure. In an evaluation of consumer complaints related to
3589 aspartame by the Centers for Disease Control and Prevention (CDC, 1984) approximately 15% of the
3590 anecdotal complaints were assigned to allergic-dermatologic reactions attributed to aspartame
3591 ingestion, such as rashes, sore throat/mouth, swelling and itching. Furthermore, case reports exist of
3592 urticaria (Kulczycki, 1986) and granulomatous panniculitis (Novick, 1985, McCauliffe and Poitras,
3593 1991) which were all thought to be related to aspartame and were previously considered by the SCF
3594 (2002).

3595 Studies by Szucs *et al.* (1986) concluded that, neither by *in vitro* methods nor by skin prick testing, *in*
3596 *vivo* aspartame was found to induce a direct degranulation of mediators from mast cells or basophiles
3597 which will result in allergy-type reactions.

3598 Garriga *et al.* (1991) concluded that after a single blind and double blind placebo controlled food
3599 challenge with aspartame exposures of up to 2000 mg none of the clinically evaluated and
3600 subsequently challenged subjects exhibited clear reproducible adverse reactions to aspartame. Geha *et*
3601 *al.* (1993) conducted a multi-centre placebo-controlled clinical study to evaluate individuals who had
3602 experienced clinical signs associated with allergy. In a double-blind crossover study, recruited subjects
3603 with a likely history of hypersensitivity to aspartame were exposed to aspartame and placebo. The
3604 authors concluded that aspartame and its conversion products were no more likely than placebo to
3605 cause allergic symptoms in the subjects.

3606 More recently, some case reports have been published (Jacob and Stechschulte, 2008, Hill and Belsito,
3607 2003) in which associations are made between aspartame intakes, in particular the subsequent
3608 exposure to the aspartame metabolite formaldehyde, and Type IV Delayed Type Hypersensitivity
3609 (DTH) reactions in patients with proven contact sensitization to formaldehyde. However, it is not
3610 possible to establish the associations observed in these two case studies with only a limited number of
3611 patients, larger case studies are needed with double-blind placebo-controlled challenges using
3612 aspartame and placebo exposures, and should include well-defined control patient groups.

3613 Although anecdotal reports in the early 1980s suggested that aspartame might be associated with
3614 allergic-type reactions, several clinical studies have shown that when the allergic-type reactions raised
3615 in these case reports were evaluated under controlled conditions aspartame is no more likely to cause
3616 reactions than placebo. The Panel concluded that the weight-of-evidence demonstrates that aspartame
3617 is not associated with allergic-type reactions in experimental models or humans.

3618 **3.2.7.9. Anecdotal reports on aspartame**

3619 Whilst acknowledging that these data exist, the Panel noted that the data do not meet the pre-specified
3620 inclusion/exclusion criteria. To ensure a comprehensive risk assessment the Panel examined the recent
3621 assessment of anecdotal reports by the EFSA National Expert Group (EFSA, 2010a).

3622 The EFSA National Expert Group assessed anecdotal data of spontaneous reports of cases with
3623 symptoms imputed to be related to aspartame (EFSA, 2010a). The anecdotal reports explored were
3624 largely self-reported, and the information was not structured and was without medical confirmation.
3625 This resulted in an incomplete database with limited possibilities to carry out meaningful analyses.
3626 The total number of cases considered was 1135. The number of cases where symptoms were classified
3627 was 1059. The case reports consisted of reports published in peer reviewed journals and reports
3628 compiled by Dr H.J. Roberts and published under the title 'Aspartame Disease – An ignored
3629 Epidemic' (Roberts, 2001). Of the total number of reports from the latter book entered in the database,
3630 689 were female, 299 were male and 77 were unclassified (the gender was not specified). Forty-three
3631 cases were children and an additional 154 cases were documented with the index case (i.e. friends and
3632 relatives).

3633 The total number of symptoms reported from all sources was 4,281, as most cases reported more than
3634 one symptom. Headache was the most frequently reported adverse effect (28.5%), followed by
3635 dizziness and giddiness (19.2%). Although the results of a questionnaire-based study (Lipton *et al.*,
3636 1989) and two double-blind out-patient investigations (Koehler and Glaros, 1988; Van den Eeden *et*
3637 *al.*, 1994) employing daily doses of up to 30 mg/kg bw/day indicated a potential association between
3638 aspartame intakes and headache, it is still not possible to deduce causality as the effect of diet has not
3639 been adequately controlled for and the interpretation of the data was complicated by a high dropout
3640 rate and a limited experimental design.

3641 The Panel noted that the number of cases is low when compared with the widespread use and that the
3642 effects were mild to moderate.

3643 **4. Discussion of aspartame toxicity database**

3644 This section discusses the toxicity database on aspartame and undertakes a hazard identification and
3645 characterisation of key end points, critical studies and points of departure for derivation of the ADI. It
3646 also provides a rationale for the description of the toxicity databases on methanol and DKP, which
3647 summarise relevant data and a hazard identification, and characterisation for their integration into the
3648 risk characterisation in Section 13.

3649 Aspartame is the methyl ester of the dipeptide of the amino acids aspartic acid and the essential amino
3650 acid phenylalanine. After oral ingestion, aspartame is fully hydrolysed, either within the lumen of the
3651 gastro-intestinal (GI) tract, or within the mucosal cells lining the inside of the GI-tract. The products
3652 that result from these reactions are methanol and the amino acids aspartic acid and phenylalanine.
3653 Hydrolysis of aspartame releases a corresponding 10% by weight of methanol. Hydrolysis is very
3654 efficient and the amount of aspartame that enters the bloodstream has been reported as undetectable in
3655 several studies conducted among others in rats, dogs, monkeys and humans (Oppermann, 1984;
3656 Burgert *et al.*, 1991). Further studies conducted in monkeys and pigs have shown that also the
3657 potential intermediate metabolite phenylalanine methyl ester is rapidly broken down to phenylalanine
3658 and methanol in the intestinal lumen (Burton *et al.*, 1984; Burgert *et al.*, 1991).

3659 The Panel concluded that the products formed following hydrolysis of aspartame are normal
3660 constituents of the diet and are metabolised by endogenous metabolic pathways. The Panel noted that
3661 there were acute intravenous toxicity studies in rat and dog at doses of 100 mg aspartame (approx 500
3662 mg/kg bw in a 200 g rat and 10 mg/kg in a 10 kg beagle dog) with no effects. The Panel concluded
3663 that these data would provide reassurance over potential systemic exposure to aspartame if it was
3664 possible to administer doses of aspartame that were not fully hydrolysed in the gastrointestinal tract
3665 (complete breakdown has been observed with no detectable plasma aspartame at doses up to the
3666 highest tested of 200 mg/kg bw).

3667 Overall, the Panel noted that no unchanged aspartame was identified in body fluids or in tissues from
3668 experimental animals or humans in any of the studies. Therefore, the Panel concluded that after oral
3669 ingestion, aspartame was hydrolysed in the gastrointestinal tract to yield aspartic acid, phenylalanine
3670 and methanol. These metabolites are then absorbed and enter normal endogenous metabolic pathways.
3671 In humans, heterozygous for phenylalanine hydroxylase mutations showed a somewhat reduced
3672 capacity compared to normal individuals to metabolise phenylalanine including that derived from the
3673 aspartame molecule. Individuals homozygous for phenylalanine hydroxylase mutations (PKU patients)
3674 have a markedly reduced capacity for phenylalanine metabolism.

3675 Several acute and subchronic toxicity, genetic toxicity, chronic toxicity and carcinogenicity and
3676 reproductive and developmental toxicity studies were made available for re-evaluation to EFSA
3677 following a public call for data that was launched in 2011 (see
3678 <http://www.efsa.europa.eu/en/dataclosed/call/110601.htm>). The Panel was aware that many of these
3679 reports would have been available at the time of the JECFA and SCF evaluations. The present
3680 evaluation by the Panel of the biological and toxicological data includes the assessment of all these
3681 unpublished studies, together with the published literature identified up to the end of November 2012.
3682 The Panel noted that the majority of these studies were performed in the period 1970-1978 and thus
3683 not performed according to Good Laboratory Practice (GLP) and OECD guidelines
3684 (<http://www.oecd.org/chemicalsafety/testingofchemicals/oecdseriesonprinciplesofgoodlaboratorypracticelndcomplianceandmonitoring.htm>). However, the Panel concluded that although the studies were
3685 old and were not performed according to current standards, they should not *per se* be disqualified and
3686 should be included in the risk assessment of aspartame. As long as the design of such studies and the
3687 report of the data were appropriate, the Panel agreed that the study should be considered in the re-
3688 evaluation of the sweetener. These studies appeared to be the basis for deriving the ADI by JECFA
3689 and SCF, using the NOAELs of 4000 mg/kg bw/day identified in the chronic and carcinogenicity
3690 studies in rats and applying an uncertainty factor of 100. Due to perceived concerns over the safety of
3691

3692 aspartame, the Panel regarded aspartame as a special case and looked again at the studies available to
3693 JECFA and the SCF.

3694 The acute toxicity of aspartame was tested in mice, rats, rabbits and dogs and was found to be very
3695 low. Similarly, sub-acute and sub-chronic studies did not indicate any significant toxic effects in rats,
3696 mice or dogs over dosing periods up to 9 weeks.

3697 Abhilash *et al.*, (2011, 2013) treated male Wistar rats with aspartame in water by gavage, daily for 6
3698 months. They found slight increases (1.50, 1.84, 1.11 and 4.03-fold increase, respectively) in serum
3699 liver enzyme (ALAT, ASAT, ALP and γ GT) levels in animals exposed to 1000 mg/kg bw/day. The
3700 Panel considered these increases as relatively low and likely to be within the normal historical range
3701 seen in many laboratories. Therefore, they were not considered to be of biological relevance.
3702 Furthermore, the Panel considered the focal infiltration of the liver by inflammatory cells, which is
3703 generally also present in the liver of control animals, as normal background pathology. Moreover,
3704 these effects were not reported in the chronic toxicity and carcinogenicity studies in both rats and
3705 mice, which were performed with much higher doses (up to 8000 mg/kg bw/day).

3706 Aspartame has been tested for genotoxicity in a number of *in vitro* and *in vivo* studies. The Panel
3707 concluded that the *in vitro* genotoxicity data on bacterial reverse mutation exhibited some limitations
3708 (e.g. absence of TA102 and WP2 uvrA *Escherichia coli*). However, the Panel considered the weight-
3709 of-evidence was sufficient to conclude that aspartame was not mutagenic in bacterial systems.
3710 Concerning mammalian systems *in vitro*, the Panel concluded that, apart from the valid UDS study
3711 that was negative, no conclusion could be drawn at the gene and chromosomal level because no
3712 studies dealing with these endpoints were available.

3713 *In vivo*, the majority of investigations on systemic genotoxicity reported negative findings. Equivocal
3714 findings were only described in a NTP study, positive in female but not in male p53 haploinsufficient
3715 mice; in two other transgenic mouse strains the results were negative.

3716 Concerning the possible site of first contact effects *in vivo*, limited data are available. However, the
3717 available *in vitro* data do not indicate a direct genotoxic activity of aspartame that might predispose to
3718 a site of first contact effect *in vivo*.

3719 Overall, the Panel concluded that the available data do not indicate a genotoxic concern for aspartame.

3720 The results from three chronic toxicity and carcinogenicity studies in rats (E33-34, 1973; E70, 1974;
3721 Ishii *et al.*, 1981) and in mice (E75, 1974) revealed no compound-related increase in neoplasms at all
3722 doses. In the rat study E33-34 (1973), in which the highest dose tested was 8000 mg/kg bw/day renal
3723 changes (pigment deposits, focal tubular degeneration and focal tubular hyperplasia) were recorded in
3724 males. In an additional two-year chronic toxicity and carcinogenicity study in Wistar rats (Ishii *et al.*,
3725 1981; Ishii, 1981) there was a dose-dependent depression of body weight gain at 2000 and 4000 mg/kg
3726 bw/day, and this effect was correlated with decreased feed consumption. Furthermore a dose-related
3727 increase in focal mineralisation of the renal pelvis in both males and females, associated with a dose-
3728 related increase in urinary calcium was recorded but this common lesion in rats attributable to mineral
3729 imbalance (refs to be added) was considered by the Panel of minimal toxicological significance. The
3730 Panel derived a NOAEL of 4000 mg/kg bw/day in these four studies (E33-34, 1973; E70, 1974; E75,
3731 1974; Ishii *et al.*, 1981), which was the highest dose level tested in three out of the four studies (E70,
3732 1974; E75, 1974; Ishii *et al.*, 1981).

3733 The results of the three studies in rats did not provide any consistent evidence of an intracranial
3734 tumourigenic effect, but showed a study-to-study variability in the incidence of such neoplasms in
3735 controls. The incidences of naturally, spontaneous, brain tumours in Sprague-Dawley derived rats
3736 have been reported by Ward and Rice (1982) to vary from 0% up to 3.3%. A similar incidence was
3737 reported by Weisburger *et al.* (1981). The Panel noted that the incidences observed in the studies with
3738 aspartame were within the range of spontaneous brain tumours observed in CD-rats. JECFA also

3739 evaluated these data and reported that the incidence of intracranial neoplasm did neither seem to
3740 predominate in any treated group nor did any particular type of neoplasm. Therefore, JECFA
3741 concluded that aspartame did not cause brain tumours in rats (JECFA, 1980). The Panel agreed with
3742 this conclusion.

3743 The US NTP carried out several 9-month carcinogenicity studies with aspartame in genetically
3744 modified Tg.AC hemizygous, p53 haploinsufficient and Cdkn2a deficient mice (NTP, 2005).
3745 According to NTP, there was no evidence of treatment-related neoplastic or non-neoplastic lesions in
3746 any of these studies. The former AFC Panel independently evaluated these studies and agreed with this
3747 conclusion (EFSA, 2006). The Panel also agreed with these conclusions but noted that the three mouse
3748 models remain at an experimental stage and had not been formally validated as experimental models
3749 for carcinogenicity testing. Therefore, the Panel did not use the results from the NTP study to derive a
3750 NOAEL.

3751 Since the last evaluation of aspartame by SCF Soffritti and co-workers reported new long term
3752 carcinogenicity studies on aspartame in both rats (Soffritti *et al.*, 2006, 2007) and mice (Soffritti *et al.*,
3753 2010). The two rat studies have already been evaluated by the former AFC Panel (EFSA, 2006) and
3754 the ANS Panel (EFSA, 2009a), respectively. Both Panels considered that the two ERF rat studies had
3755 flaws that brought into question the validity of the findings, as interpreted by the ERF. In particular,
3756 the high background incidence of chronic inflammatory changes in the lungs and other vital organs
3757 and tissues and the uncertainty about the correctness of the diagnoses of some tumour types were
3758 major confounding factors in the interpretation of the findings of the study. The ANS Panel also noted
3759 that the increase in incidence of mammary carcinoma was not considered indicative of a carcinogenic
3760 potential of aspartame since the incidence of mammary tumours in female rats is rather high and varies
3761 considerably between carcinogenicity studies. The ANS Panel noted that the only consistent findings
3762 reported by the authors in the two rat studies were an increased incidence of lymphomas/leukaemias in
3763 female rats and an increased trend or incidence of lymphomas/leukaemias in treated males and females
3764 at the high dose group (EFSA, 2009a).

3765 In the mouse study (Soffritti *et al.*, 2010) Swiss mice were given aspartame (up to 4000 mg/kg
3766 bw/day) in the feed from pre-natal life (12 days of gestation) until death. The authors of the study
3767 reported a dose-related increase in the incidence of hepatocellular carcinomas in male mice, and an
3768 increase in the incidence of alveolar/bronchiolar carcinomas in males of the highest dose group,
3769 whereas no compound-attributed carcinogenic effects were reported in female mice. The former ANS
3770 Panel (EFSA, 2011a) and EFSA (EFSA, 2011b) observed that the hepatic and pulmonary tumour
3771 incidences reported by Soffritti *et al.* (2010) all fall within their own historical control ranges for
3772 spontaneous tumours. It was also noted that Swiss mice are known to have a high background
3773 incidence of spontaneous hepatic and pulmonary tumours (Prejean *et al.*, 1973; Fox *et al.*, 2006).

3774 The validity of the ERF studies has been questioned by several investigators, and more recently in an
3775 US EPA (US Environmental Protection Agency) commissioned NTP report (NTP-EPA, 2011). For
3776 instance, Cruzan (2009) argued that lung infections probably played a role in the development of what
3777 Soffritti *et al.* (2002) referred to as lympho-immunoblastic lymphomas. The Panel noted that the same
3778 arguments are applicable to the Soffritti *et al.* (2006) and Soffritti *et al.* (2007) studies and considered
3779 it plausible that lung infections might have played a role in the development of neoplastic changes that
3780 originated from mediastinal lymph nodes. Similarly, Schoeb *et al.* (2009) and Schoeb and McConnell
3781 (2011) assessed the likelihood that lifetime cancer bioassays of aspartame, methanol, and methyl
3782 tertiary butyl ether conducted with conventional (not specific pathogen free) Sprague-Dawley rats
3783 were compromised by *Mycoplasma pulmonis* disease. The authors concluded based on published
3784 reproductions of the lesions (Belpoggi *et al.*, 1999, Soffritti *et al.*, 2005) that the accumulation of
3785 lymphocytes, plasma cells, and neutrophils in the lungs of the rats used in these studies was more
3786 plausibly due to *M. pulmonis*, possibly with contributions by other pathogens, than to a rare cellular
3787 type of lymphoma having an organ distribution uncharacteristic of recognised forms of lymphoma in
3788 rats (Schoeb *et al.*, 2009). The Committee on Carcinogenicity of Chemicals in Food Consumer
3789 Products and the Environment (COC) also evaluated the Soffritti *et al.* (2006) study on aspartame

3790 (COC, 2006). In light of the limitations in the design of this study and the use of animals with a high
3791 infection rate, the COC considered that no valid conclusions could be drawn from this study. Schoeb
3792 and McConnell (2011) subsequently confirmed the occurrence of inflammatory lesions in not only the
3793 respiratory tract but also lymph nodes, thymus, pleura and brain, suggesting the involvement of other
3794 pathogens in addition to *M. pulmonis*. They furthermore stated that upon screening of the ERF animal
3795 colony, antibodies to ‘mycoplasma’ had been found. Overall, Schoeb and McConnell (2011)
3796 concluded that the reported development of lymphomas in the ERF studies performed with Sprague-
3797 Dawley rats should not be taken into account in cancer risk assessments. The Panel agreed with the
3798 above conclusions.

3799 Finally, the ANS Panel noted that an NTP review of ERF studies on methanol, methyl-*t*-butyl-ether,
3800 ethyl-*t*-butyl-ether, acrylonitrile and vinyl chloride had been performed following a request by US
3801 EPA (NTP-EPA, 2011). In the case of the methanol study (Soffritti *et al.*, 2002), the NTP pathologists
3802 did not confirm the diagnoses of malignancy and reported fewer lymphoid neoplasms, mainly of the
3803 respiratory tract. They also observed fewer neoplasms of the inner ear and cranium and noted that
3804 there was chronic inflammation of the nasal cavity, ear canal, trachea, and lung, indicating infection of
3805 the animals by one or more respiratory pathogens. EPA concluded that many of the malignant
3806 neoplasms and the lymphoid dysplasias diagnosed by the ERF pathologists were cases of hyperplasia
3807 related to chronic infection (NTP-EPA, 2011).

3808 The reproductive and developmental toxicity studies on aspartame comprised an embryotoxicity and
3809 teratogenicity study performed in the mouse, a two-generation toxicity study in the rat, five perinatal
3810 and postnatal developmental studies in the rat, a reproductive performance and developmental study in
3811 the rat and an embryotoxicity and teratogenicity study in the rat. In addition, eight embryotoxicity and
3812 teratogenicity studies were performed in the rabbit, four with administration of aspartame by diet and
3813 four by gavage.

3814 The Panel identified a NOAEL for developmental toxicity in the mouse (E89, 1975) of 5700 mg/kg
3815 bw/day, the highest dose level tested. In the case of the rat, the two-generation study was reported very
3816 sparsely. However, the Panel considered this study (E11, 1971) together with other segment I and
3817 segment III studies that included a full examination of the F2A pups from the two-generation study
3818 (E9, 1972) sufficient to fulfil the requirements of a two-generation reproduction study. The animals
3819 received aspartame in the diet at dose levels of 0 (control), 2000 and 4000 mg/kg bw/day. Statistically
3820 significant pup body weight suppression and smaller size at weaning were recorded in the 4000 mg/kg
3821 bw/day. The Panel identified a NOAEL from these studies of 2000 mg/kg bw/day based on the lower
3822 pup weights at weaning in both generations (E9, 1972; E11, 1971).

3823 Further rat studies included a male and female fertility study (E10, 1972) and three segment peri- and
3824 post-natal developmental studies (E39, 1973; E47, 1973; E48, 1973). In all these studies, the route of
3825 aspartame administration was via the diet at dose levels of 0 (control), 2000, or 4000 mg/kg bw/day.
3826 The effects on the pups were depressed body weights at birth and a decreased survival rate, which
3827 were observed at the high dose only. For all these studies the Panel derived a NOAEL of 2000 mg/kg
3828 bw/day. In an embryotoxicity and teratogenicity study (E5, 1970), no evidence of treatment-induced
3829 fetopathological effects was observed, and the Panel derived from this study a NOAEL of 4000 mg
3830 aspartame/kg bw/day, the highest dose level tested, for developmental and maternal toxicity.

3831 In a peri- and post-natal development study (E49, 1973) in rats, the effects of aspartame (4000 mg/kg
3832 bw/day) were compared with those of L-phenylalanine (1800 mg/kg bw/day) or L-aspartic acid (1700
3833 mg/kg bw/day) or the combination of L-phenylalanine and L-aspartic acid (2100 and 1800 mg/kg
3834 bw/day). The intention of the study was to have equimolar doses of each substance in the diet. The
3835 authors of the study reported a significant depression in body weight in the aspartame, L-
3836 phenylalanine and L-phenylalanine + L-aspartic acid groups as compared to the control group and
3837 concluded that L-phenylalanine on its own or in combination with aspartic acid decreased maternal
3838 and pup body weight and therefore duplicated the observed effects of aspartame on these endpoints.
3839 The Panel agreed with the author’s conclusion but noted the poor survival of control pups.

3840 The Panel noted that the two-generation reproduction toxicity study (E11, 1971) was not reported
3841 according to current standards. However, when taken together with the segment I study (E10, 1972)
3842 and segment III studies (E47, 1973; E48, 1973) the Panel considered the available data sufficient to
3843 conclude on reproductive toxicity. In summary, the results of the reproductive and developmental
3844 toxicity studies in rats indicate NOAELs that ranged from 2000 mg aspartame/kg bw/day (E11, 1971;
3845 E39, 1973; E47, 1973; E48, 1973) to 4000 mg/kg bw/day (E5, 1970; E9, 1972; E10, 1972). The Panel
3846 noted that where developmental changes in pup body weight were observed at birth in studies at the
3847 dose of 4000 mg/kg bw/day, these might be attributed to a combination of malnutrition and nutritional
3848 imbalance as a consequence of excessive intake of phenylalanine, a breakdown product of aspartame.
3849 In support of this hypothesis, the Panel noted that administration of a dose of L-phenylalanine (1800
3850 mg/kg bw/day) that was equimolar to 4000 mg aspartame/kg bw/day led to a similar decrease in
3851 maternal and pup body weight as observed in a concurrent aspartame group (E49, 1973). Furthermore,
3852 supplementation of the diet with L-phenylalanine (5-7%) (Kerr and Waisman, 1967) or the
3853 combination of 3% L-phenylalanine and 0.5% α -methylphenylalanine (Brass *et al.*, 1982) has been
3854 shown to result in severe nutritional imbalance in rats (Kerr and Waisman, 1967) and to lead to a
3855 reduction in pup body weight at birth (Brass *et al.*, 1982). In addition, milk production in mothers
3856 exposed to excessive L-phenylalanine intake has been reported to be decreased, which could further
3857 contribute to malnutrition of the pups during weaning and result in pup mortality (Boggs and
3858 Waisman, 1962; Kerr and Waisman, 1967).

3859 The Panel considered that the depression in pup body weight in the high dose groups might be
3860 accounted for by the effect of high exposure to phenylalanine from metabolism of aspartame and it
3861 should be regarded as an (indirect) effect related to the test compound. The Panel noted that milk
3862 quality in the rat could be adversely affected by high levels of plasma phenylalanine and that body
3863 weights of pups could have been adversely affected (decreased) by decreased milk quality. This might
3864 provide a plausible explanation for the effects of aspartame on pup body weight at weaning.

3865 In several reproductive and developmental toxicity studies, aspartame was administered to rabbits via
3866 the diet (E53, 1973; E54, 1974; E55, 1973; E62, 1973; E63, 1973). The Panel noted that the actual
3867 doses of aspartame to which the rabbits were exposed did not exceed 1880 mg/kg bw/day (range 1160
3868 – 1870 mg/kg bw/day when the intended dose was 4000 mg aspartame/kg bw/day; 670 – 1880 mg/kg
3869 bw/day when the intended dose was 2000 mg aspartame/kg bw/day) due to the considerable decrease
3870 in feed intake observed in the does (by 32-62%) in this dose group. In another series of pre-natal
3871 developmental studies, rabbits were exposed to aspartame (at doses up to 2000 mg/kg bw/day) by
3872 gavage (E51, 1973; E52, 1973; E79, 1974). Overall, the Panel considered that the data from the
3873 studies described above were confounded both by the decrease in feed intake (when aspartame was
3874 administered via the diet or by gavage), the poor health of the animals, and, in many cases, by a
3875 number of deaths of pregnant rabbits in the treated groups possibly related to misdosing via the gavage
3876 technique.

3877 In a separate study, pregnant rabbits were dosed by gavage at levels of 0 (control), 500, 1000 and 2000
3878 mg/kg bw/day (E90, 1975). This study also included L-phenylalanine and L-aspartic acid groups at
3879 dose levels equimolar to the top dose of 2000 mg aspartame/kg bw. An approximately 65% and 40%
3880 decrease in feed consumption was observed in the 2000 mg aspartame/kg bw/day group and the L-
3881 phenylalanine treated animals, respectively, which in the high dose aspartame group was accompanied
3882 by a significant body weight loss. Twenty-four abortions were observed in the high dose aspartame
3883 group compared to no abortions in controls and four in the L-phenylalanine group. Mean fetal body
3884 weight and length were significantly reduced in both the 2000 mg/kg bw/day group and the L-
3885 phenylalanine group animals and a significantly higher rate of total (major and minor) malformations
3886 in the 2000 mg aspartame/kg bw/day group animals compared to the concurrent control group was
3887 reported. The authors of the study suggested that the effects observed at 2000 mg/kg bw/day and to a
3888 lesser extent in the L-phenylalanine group appeared to be produced by a severe reduction of nutrient
3889 intake.

3890 The Panel concluded that despite this plausible explanation, a direct toxic effect of aspartame could
3891 not be ruled out. Although pregnant female rabbits from the high-dose group in study E90 (1975)
3892 manifested body weight loss and low, to very restricted feed consumption, the Panel was unable to
3893 determine that the reported developmental effects on fetus were due to a reduced nutrient intake by the
3894 mother, as suggested by the authors. The Panel also considered the possibility that exposure to high
3895 levels of phenylalanine resulting from metabolism of aspartame may be in part responsible for these
3896 effects in the high dose aspartame group because similar effects, although less severe, were seen in the
3897 phenylalanine group. However, the Panel noted that the dose of L-phenylalanine administered was
3898 25% lower than the animals would have received as phenylalanine from the high dose of 2000 mg
3899 aspartame/kg bw/day. Based on the above considerations the Panel identified a NOAEL of 1000 mg
3900 aspartame/kg bw/day for maternal (weight loss) and developmental toxicity (weight loss and
3901 malformations) in study E90 (1975).

3902 The Panel agreed that the results in developmental rabbit studies with aspartame may have been
3903 influenced by the nutritional status of the female rabbits as a result of gastrointestinal disturbances and
3904 the potential effect of phenylalanine from metabolism of the test compound. Rabbits are known to be
3905 susceptible to disturbances of the gastrointestinal tract. Gastrointestinal disturbances and lower feed
3906 intake in the rabbits may result from administering high levels of test preparation by gavage and may
3907 lead to disturbed clinical appearance, death or abortions. Similar effects have been reported in
3908 pregnant rabbits following administration of sucralose (SCF, 2000), steviol glycosides (EFSA, 2010b)
3909 and neotame (EFSA 2007). The Panel further noted that the gastrointestinal effects in the rabbit
3910 studies may be species specific and would therefore not be relevant for humans. On the other hand, the
3911 rabbits may exhibit gastrointestinal effects as secondary effects prior to abortion, which is a primary
3912 effect, caused by toxicity of a test compound to the mother or fetus (Cappon *et al.*, 2005; Matzuoka *et*
3913 *al.*, 2006). Based on the available descriptions of the developmental rabbit studies the Panel was
3914 unable to ascertain whether the gastrointestinal disturbances were a cause of or a consequence of the
3915 reproductive and developmental effects seen. Overall, the Panel concluded that the rabbit studies
3916 should not be considered as pivotal in the safety evaluation of aspartame because of the confounding
3917 factors affecting these studies.

3918 In summary, the highest NOAEL for developmental toxicity in rabbits of 2400 mg aspartame/kg
3919 bw/day was identified by the Panel in study E63, in which the test compound was administered in the
3920 diet. The Panel identified a NOAEL of 1000 mg aspartame/kg bw/day for maternal (weight loss) and
3921 developmental toxicity (weight loss and malformations) in study E90 (1975). The Panel noted that the
3922 severely decreased feed intake could have a negative effect on the nutritional status of these females
3923 and the observed adverse effects could result from gastrointestinal disturbances as well as from a
3924 potential direct toxic effect of phenylalanine.

3925 The Panel noted that there were limitations and deficiencies in the reproductive and developmental
3926 toxicity database in rats and rabbits. In many cases, these were likely to be attributable to study
3927 methodology and reporting requirements at the time when the studies were conducted. The Panel
3928 recognised the difficulty to evaluate retrospectively whether the reported effects would account for the
3929 toxicity of aspartame and thus be of toxicological relevance. The Panel considered whether this could
3930 be resolved by performing reproductive and developmental toxicity studies according to current
3931 guidelines. The Panel noted that this would require doses equivalent to if not higher than those used in
3932 the earlier studies. For animal welfare reasons, modern studies would not generally be conducted at
3933 doses greater than the lower end of the identified NOAEL range; the Panel therefore concluded that it
3934 was unlikely that a definitive modern study would be possible or ethical. The Panel considered that the
3935 attribution of effects to phenylalanine was both plausible and supported by the data on metabolism and
3936 kinetics of aspartame. The Panel considered that data on adverse effects of plasma phenylalanine on
3937 pregnancy outcome in phenylketonuria sufferers could be helpful. The Panel considered that
3938 combining these data with information on toxicokinetics of phenylalanine derived from aspartame
3939 might allow the identification of chemical specific adjustment factors that could be used in place of
3940 default uncertainty factors.

3941 Epidemiological studies have explored possible associations of aspartame, or artificial sweeteners
3942 more broadly, with pre-term delivery and various cancers. The epidemiological data on aspartame
3943 were previously reviewed by SCF (2002). The Panel considered and agrees with the conclusions of
3944 SCF that there was no evidence for adverse effects of aspartame in the human population. The new
3945 epidemiology studies, published since the SCF opinion, are described below.

3946 A large and well conducted prospective cohort study in Denmark found a significantly elevated risk of
3947 pre-term delivery (particularly medically induced pre-term delivery) in women with higher reported
3948 consumption of artificially sweetened drinks (but not aspartame specifically) at about 25 weeks
3949 gestation. However, even in the highest exposure category (≥ 4 servings per day), odds ratios were less
3950 than two, and in the absence of any identified plausible underlying toxic mechanism or of independent
3951 replication in other studies, it is possible that the finding resulted from a combination of uncontrolled
3952 residual confounding and chance.

3953 To explore whether the findings of the Danish study could be replicated, another prospective study in
3954 Norway investigated the relation between consumption of artificially sweetened and sugar-sweetened
3955 soft drinks during the first 4-5 months of pregnancy and subsequent pre-term delivery in a large cohort
3956 of Norwegian women. No significant trends were found in the risk of pre-term delivery with
3957 increasing consumption either of artificially sweetened drinks or of sugar-sweetened drinks. Small
3958 elevations of risk were observed with higher consumption of artificially sweetened soft drinks, but
3959 after adjustment for covariates, these reached statistical significance only when categories of
3960 consumption were aggregated to four levels, and then the odds ratio for the highest category (≥ 1
3961 serving/day) was only 1.11 (95% CI 1.00-1.24) in comparison with never consumption. This was
3962 driven by an increase in spontaneous but not medically induced pre-term delivery. Associations with
3963 sugar-sweetened soft drinks tended to be somewhat stronger, with an adjusted odds ratio of 1.25 (95%
3964 CI 1.08-1.45) for consumption of at least one serving per day.

3965 This pattern of results contrasts with that reported by the Danish study. The association of pre-term
3966 delivery with artificially sweetened soft drinks was much weaker and barely discernible, applied more
3967 to spontaneous than medically induced deliveries, and was exceeded by an association with
3968 consumption of sugar-sweetened soft drinks. The Panel noted that effects may have been
3969 underestimated because of non-differential inaccuracies in the assessment of dietary exposures, but the
3970 method was similar to that used by the Danish study, and the same for sugar-sweetened as for
3971 artificially sweetened soft drinks.

3972 When findings from the two studies are considered together, they do not point clearly to a hazard. In
3973 summary, both studies appear to have been well designed and conducted. Noting this, the Panel
3974 concluded that even at high level of exposure to artificially sweetened soft drinks the risk of pre-term
3975 delivery is likely to be small. This small risk could be a consequence of uncontrolled residual
3976 confounding, and the inconsistencies in the patterns of association reinforce this uncertainty. The
3977 Panel noted that the studies only allowed conclusion on artificially sweetened soft drinks and not on
3978 aspartame specifically.

3979 More recently, a study by Schernhammer *et al.* (2012) examined the risk of lymphatic and
3980 haematopoietic cancers in relation to consumption of diet soda and aspartame sweeteners added at the
3981 table was examined, in two US cohorts. The authors reported that the category of highest aspartame
3982 intake (≥ 143 mg/day) was associated with a significantly elevated relative risk of Non Hodgkin
3983 Lymphoma (NHL) (1.64, 95%CI 1.17-2.29) and of multiple myeloma (3.36, 95%CI 1.38-8.19) in
3984 men. However, there was no consistent trend in risk with increasing exposure, and there were no
3985 corresponding elevations in risk in women. No clear association with leukaemia was apparent in either
3986 men or women. The Panel noted that the positive findings could be given little weight, given their
3987 limitation to men, the small relative risks observed, and the lack of clear dose-response relationships.
3988 The authors' proposed explanation for the differential associations in men and women is unconvincing
3989 in the absence of data on other more important dietary sources of methanol.

3990 A Swedish case-control study of malignant brain tumours suggested a possible link with consumption
3991 of low-calorie drinks, most of which contained aspartame. However, the association was not
3992 statistically significant, and a more rigorous prospective cohort study in the United States found that
3993 risk of malignant glioma was significantly lower in subjects with higher intakes of aspartame at
3994 baseline. Moreover, a case-control study of children with medulloblastoma/primitive neuroectodermal
3995 tumour, also in the United States, found only a weak and statistically non-significant association with
3996 mothers' recalled consumption of low-calorie carbonated drinks during pregnancy.

3997 A cohort study of haematopoietic cancer in the United States showed no relation to consumption of
3998 aspartame, and in a set of linked case-control studies in Italy, associations between consumption of
3999 sweeteners other than saccharin (mostly aspartame) and cancers of the oral cavity and pharynx,
4000 oesophagus, colon, rectum, larynx, breast, ovary, prostate and kidney were unremarkable.

4001 In a small pilot case-control study on brain cancer in adults conducted in southern France, 122 cases
4002 were compared to 122 controls with other neurological diagnoses (Cabaniols *et al.*, 2011). There was
4003 no association with aspartame consumption during the past five years of at least once per week (OR
4004 1.02, 95%CI 0.57-1.85). However, the Panel noted that, due to different study limitations, the non-
4005 positive finding provides little reassurance of an absence of hazard.

4006 Overall, the reassurance of safety that is provided by non-positive findings in these epidemiological
4007 investigations is limited by (sometimes unavoidable) shortcomings in their design, and particularly in
4008 their assessment of exposures. At the same time, the studies do not point strongly to any hazard, and
4009 do not provide a suitable point of departure for derivation of a toxicological reference value.

4010 Magnuson *et al.* (2007) and Weihrauch and Diehl (2004) reviewed strengths and weaknesses of these
4011 four cancer epidemiological studies and concluded that there was no evidence that aspartame posed a
4012 carcinogenic risk. The National Expert meeting of the EFSA Advisory Forum (EFSA, 2010a) also
4013 concluded that there was no evidence to support an association between aspartame and brain, or
4014 haematopoietic or other tumours prevalence. The Panel agrees with these conclusions.

4015 **5. Biological and toxicological data on methanol**

4016 The section contains summarised information on methanol metabolism and kinetics and a few detailed
4017 descriptions of the studies on release of methanol from aspartame.

4018 **5.1. Absorption, distribution, metabolism and excretion of methanol**

4019 Methanol is converted to formaldehyde through one of at least four pathways, involving alcohol
4020 dehydrogenase, catalase, CYP2E1 or a Fenton-like system (Fe(II)/H₂O₂) (Dikalova *et al.*, 2001;
4021 Sweeting *et al.*, 2010; MacAllister *et al.*, 2011). Primates including humans metabolise methanol
4022 primarily using the alcohol dehydrogenase pathway whereas rodents appear to use all four pathways.
4023 In monkeys, about 25% of the ¹⁴C from [¹⁴C-methyl]-aspartame enters into transmethylation or
4024 formylation biosynthetic reactions (E92, 1976). Formaldehyde is converted to formate by a specific
4025 formaldehyde dehydrogenase, and formate is converted to carbon dioxide. In monkeys, about 70% of
4026 the ¹⁴C from [¹⁴C-methyl]-aspartame follows this path to carbon dioxide (E92, 1976). Formate can
4027 enter the one-carbon metabolic pool by formylating tetrahydrofolic acid to N⁵,N¹⁰-methylene-
4028 tetrahydrofolate.

4029 In mammals, methanol is readily absorbed following ingestion, inhalation and dermal exposure (COT,
4030 2011). Methanol can readily enter the total body water and has a volume of distribution of 0.6-0.7
4031 L/kg bw. Methanol is subject to a significant first pass metabolism. The overall metabolism of
4032 methanol proceeds by stepwise oxidation via formaldehyde to formate and then to carbon dioxide. The
4033 metabolism of formaldehyde is very efficient. Even after intravenous infusion, it was difficult to detect
4034 formaldehyde in blood, in which formaldehyde has been reported to display a half-life of about 1
4035 minute (McMartin *et al.*, 1979; Tephly and McMartin, 1984). The oxidation of formate to carbon
4036 dioxide varies between species, the rate of formate elimination in humans and non-human primates

4037 being half of that in rats (Kavet and Nauss, 1990). In rodents, formate is converted to carbon dioxide
 4038 through a folate-dependent enzyme system and a catalase-dependent pathway (Dikalova *et al.*, 2001)
 4039 whereas in humans metabolism occurs exclusively through the folate-dependent pathway (Hanzlik *et al.*,
 4040 *et al.*, 2005). Studies in humans have shown that approximately 80% of an oral [¹⁴C]formate dose
 4041 (administered as a single dose of 3.9 g calcium formate) is exhaled as ¹⁴CO₂, 2-7% excreted in urine
 4042 and approximately 10% is metabolically incorporated (Hanzlik *et al.*, 2005).

4043 Formaldehyde is an intermediate in the formation of formic acid from methanol. Even after
 4044 intravenous infusion of formaldehyde in dogs, cats, rabbits, guinea pigs and rats, it was difficult to find
 4045 this substance in the blood, because of a very short half-life (~ 1 minute). Formaldehyde formation
 4046 was studied in older publications on acute toxicity of methanol, but this intermediate could not be
 4047 detected in animals or in humans (Tephly and McMartin, 1984). In monkeys given methanol orally via
 4048 a nasogastric tube [3 g /kg bw as a 20% (w/v) solution of [¹⁴C]methanol (1300 dpm/μmol of
 4049 methanol)], formaldehyde could be detected in the blood at a level of 27- 45 μM over the period up to
 4050 18 hours following dosing with methanol. No formaldehyde could be found in liver, kidney, urine or
 4051 several neural tissues (LOD 25 μM; ~ 0.75 mg/L or 0.75 mg/kg tissue). Formic acid was readily
 4052 detected in all tissues and body fluids studied, in particular in urine, liver, blood and kidney
 4053 (McMartin *et al.*, 1979).

4054 When ¹⁴C-methanol or [¹⁴C-methyl]-aspartame were given orally to rats or monkeys in equimolar
 4055 doses, in both species, the radioactivity was rapidly eliminated via exhaled air (~60% of the dose in
 4056 rats; ~ 70% in monkeys) within 8 hours post dosing. With methanol, a slightly higher rate of
 4057 exhalation was observed, probably because hydrolysis and absorption are required when the
 4058 radioactivity was administered as labelled aspartame. Virtually no radioactivity appeared in the faeces
 4059 and 2 - 5% of the dose was eliminated via urine. In rats, about 40% and in monkeys about 30% of the
 4060 dose was not accounted for, but it is noted that residual radioactivity in the carcass from incorporation
 4061 through the 1-carbon cycle was not studied. These studies were undertaken with dose levels of 10 to
 4062 20 mg aspartame/kg bw (Oppermann, 1984).

4063 In a recent study (Lu *et al.*, 2012) formaldehyde hydroxymethyl DNA adducts have been measured
 4064 after administration of labelled [¹³CD₄]-methanol to rats (500 and 2000 mg/kg bw/day for 5 days) in
 4065 multiple tissues in a dose dependent manner. This finding is in line with the known metabolism of
 4066 methanol.

4067 The half-life for the systemic clearance of methanol has been reported to be 2.5-3 hours following oral
 4068 doses of less than 100 mg/kg bw methanol given to human volunteers, increasing to 24 hours or more
 4069 for doses greater than 1000 mg/kg (WHO, 1997). Stegink *et al.* (1981b) reported a human volunteer
 4070 study in which methanol cleared with a half life of 2.5 to 3 hours after administration for a dose of 80
 4071 mg aspartame/kg bw.

4072 **5.2. Toxicological data of methanol**

4073 **5.2.1. Acute oral toxicity**

4074 A limited number of studies have assessed the acute oral toxicity of methanol. The LD₅₀ reported were
 4075 7300 mg/kg in mice, 5628 mg/kg in rats and 7000 mg/kg in monkeys (Lewis, 1992).

4076 **5.2.2. Short-term and sub-chronic toxicity**

4077 In a 90-day study, Sprague-Dawley rats (30/sex/group) were administered 0, 100, 500, or 2500 mg
 4078 methanol/kg bw/day by gavage for 6 weeks until the day of the interim necropsy. After the interim
 4079 sacrifice, all surviving rats were dosed daily until the end of the experiment (EPA, 1986). No
 4080 differences in body weight gain, food consumption, gross or microscopic examinations were reported
 4081 between treated animals and controls. Serum alanine transaminase (ALT) and serum alkaline
 4082 phosphatase (AP) of the high-dose group's males (p≤ 0.05) and females were approximately 30% and
 4083 40% higher respectively than those of the control group at the end of the experiment. Blood Urea

4084 Nitrogen (BUN) averages in the high-dose group were 17% and 12%, respectively lower than control
4085 groups ($p \leq 0.05$). Mean Corpuscular Haemoglobin (MCH) averages in the high-dose group females
4086 were also significantly lower than controls ($p \leq 0.001$) as well as Mean Cell Volume (MCV)x
4087 averages were lower in the mid-dose and low-dose females groups ($p \leq 0.05$). Brain weights of high-
4088 dose group males and females were significantly decreased compared to control group at the end of
4089 the treatment. The authors considered these differences to be treatment-related but very small, and not
4090 associated with any histological lesion. A higher incidence of colloid in the hypophyseal cleft of the
4091 pituitary gland was also observed in the high-dose group (24/40) compared to the controls (3/40). The
4092 authors identified a NOAEL from this study of 500 mg/kg day.

4093 **5.2.3. Genotoxicity of methanol**

4094 The genotoxicity of methanol has been studied in bacterial and mammalian *in vitro* tests and in some
4095 *in vivo* tests. A brief description of the studies found in the literature, is given below and summarised
4096 in Annex H.

4097 *In vitro studies*

4098 De Flora *et al.* (1984) published a survey of 71 drinking water contaminants and their capacity to
4099 induce gene reversion in the Ames test with *Salmonella typhimurium* strains TA 98, 100, 1535, 1537,
4100 and 1538 and found methanol to be negative. De Flora *et al.* (1984) also reported methanol to be
4101 negative for induction of DNA repair in *Escherichia coli* strains WP2, WP2 (*uvrA*-, *polA*-), and
4102 CM871 (*uvrA*-, *recA*-, *lexA*-), both when tested in the presence or absence of metabolic activation. The
4103 Panel considered these studies as providing insufficient information and therefore, were not taken into
4104 further consideration.

4105 Griffiths (1981) reported a genotoxicity study of methanol using fungi, and reported methanol to be
4106 negative for the induction of aneuploidy in *Neurospora Crassa*. The Panel considered that the methods
4107 implemented were thought not to be sufficiently robust to support the results reported.

4108 Simmon *et al.* (1977) listed methanol as one of 45 chemicals that gave negative results with
4109 *Salmonella typhimurium* strains TA 98, 100, 1535, 1537 and 1538, tested with or without metabolic
4110 activation. However, for the reasons explained in detail in Annex H, the Panel considered this study
4111 provided insufficient details and therefore, was not taken into further consideration.

4112 Abbondandolo *et al.* (1980) used a *ade6-60/rad10-198,h⁻* strain of *Schizosaccharomyces pombe* (P1
4113 strain) to determine the capacity of methanol to induce forward mutations. Negative results were
4114 obtained for methanol, both without and with metabolic activation. In contrast to this finding, weakly
4115 positive results for methanol were obtained for the induction of chromosomal malsegregation in the
4116 diploid strain P1 of *Aspergillus nidulans* (Crebelli *et al.*, 1989). The disturbance of chromosome
4117 segregation by methanol was attributed by the study authors to the non-specific damage of cell
4118 membranes induced at high doses by methanol and other aliphatic alcohols. The Panel noted that the
4119 fungal systems are not considered relevant for chromosomal malsegregation induction in mammalian
4120 cells. The Panel considered that the methods implemented were sufficiently robust to support the
4121 results reported.

4122 Methanol was studied in a *Salmonella* mutagenicity test in the presence or in the absence of metabolic
4123 activation. No mutagenicity was detected in strains TA 98, 100, 1535, 1537, 1538 in 5 doses up to 3.6
4124 mg/plate (Gocke *et al.*, 1981). The bacterial reverse mutation assay followed a standard protocol of
4125 that time. However, no detailed results for methanol were reported. Furthermore, TA102 and
4126 *Escherichia coli* were not included in the study and the maximal concentration tested (3.6 mg/plate)
4127 was lower than recommended in current guidelines. The Panel considered the bacterial reverse
4128 mutation assays, which were part of the screening study by Gocke *et al.* (1981), as providing
4129 insufficient information for further consideration.

4130 NEDO (1987) reported methanol to be negative for gene mutation in *Salmonella typhimurium* TA 98,
4131 100, 1535, 1537, 1538; *Escherichia coli* WP2 uvvA (Pre-incubation, absence and presence of S9; 10,
4132 50, 100, 500, 1000, 5000 µg/plate). The Panel considered that the methods implemented were
4133 sufficiently robust to support the results reported.

4134 NEDO (1987) also used Chinese hamster lung cells to monitor the capacity of methanol to induce (a)
4135 forward mutations to azaguanine, 6-thioguanine, and ouabain resistance (15.8, 31.7, 47.4, 63.3
4136 mg/ml), (b) chromosomal aberrations (7.1, 14.3, 28.5 mg/ml) and (c) SCEs (7.1, 14.3, 28.5 mg/ml).
4137 Methanol was negative in tests for gene mutation and CA, but displayed some capacity to induce SCE
4138 in Chinese hamster lung cells, since the incidence of these lesions at the highest concentration (28.5
4139 mg/mL) was significantly greater than in controls (9.41 ± 0.416 versus 6.42 ± 0.227 [mean \pm SE per
4140 100 cells]). The Panel noted that this concentration of methanol exceeded the maximum concentration
4141 recommended for *in vitro* studies (i.e. 5 mg/mL or 5µl/mL or 10mM, whichever is the lowest) (OECD
4142 test guidelines 473 and 487.) The Panel considered that the methods implemented were not
4143 sufficiently robust to support the results reported.

4144 In a test for forward mutation in *Escherichia coli* SA500, methanol concentrations above 23 % were
4145 cytotoxic, reducing survival of the cells to less than 40 %. A 23 % methanol solution produced no
4146 mutagenic effects; cell survival was 53 % (Hayes *et al.*, 1990). However, the assay was performed
4147 with one *Escherichia coli* strain (SA 500) as well as in the absence of metabolic activation only. The
4148 Panel considered that the methods implemented were not sufficiently robust to support the results
4149 reported.

4150 Ohno *et al.* (2005) developed a genotoxicity test system based on p53R2 gene expression in human
4151 cells. The assay was based on examination of the chemical activation of the p53R2 gene, which was
4152 assessed by the incorporation of a p53R2-dependent luciferase reporter gene into two human cell lines,
4153 MCF-7 and HepG2. The Panel considered that the methods implemented were sufficiently robust to
4154 support the results reported, but not adequate for the present evaluation, because the endpoint used
4155 was not relevant for the mechanism of action of non-radiomimetic chemical compounds.

4156 McGregor *et al.* (1988) aimed to define the optimal conditions for the metabolic activation of
4157 chemicals to mutagens in the L5178Y/TK+/- Mouse Lymphoma Assay using different pro-mutagenic
4158 compounds. Methanol was assessed for its mutagenicity at 5-50 µl/ml (approximately 3.95-39.5
4159 mg/ml). Ten µl methanol/ml in the presence of 2.5 mg S9/ml exhibited a mutagenic effect, relative
4160 total growth (RTG) was reduced to less than 30%. Furthermore, increasing the concentration of the S9
4161 mix to 7.5 mg/ml reduced RTG to about 15% but increased the mutant fraction from 60 in the control
4162 to 650 mutants/10⁶ at 5 µl methanol/ml. However, the Panel noted that the lowest concentration tested
4163 (5 µl/ml) exceeded in terms of molarity (13.4 M) the maximum concentration recommended in OECD
4164 guideline 476 (5 mg/ml, 5 µl/ml, or 0.01 M, whichever is the lowest). Therefore, indirect effects due to
4165 non-physiological culture conditions at all concentrations tested cannot be excluded. The Panel
4166 considered that the methods implemented were not sufficiently robust to support the results reported.

4167 DeMarini *et al.* (1991) examined methanol in two independent microscreen prophage-induction assays
4168 with *Escherichia coli* WP2 (λ). Five methanol concentrations ranging from 0.15 up to 5% [i.e. from 37
4169 mM to 1.23 M, assuming the concentrations are expressed as % (v/v)], which included toxic levels,
4170 without and with metabolic activation, did not result in prophage-induction. The Panel considered that
4171 the methods implemented were sufficiently robust to support the results reported.

4172 Hamada *et al.* (1988) examined methanol (2-8% *viz.* 0.49-1.97 M) for induction of gene mutations in
4173 *Saccharomyces cerevisiae* ATCC26422 as a control culture within a study which aimed at elucidating
4174 the effects of ethidium bromide. Methanol did not induce gene mutations, but was only tested without
4175 a metabolic activation system. The Panel considered that the methods implemented were not
4176 sufficiently robust to support the results reported.

4177 In a survey by Shimizu *et al.* (1985) which aimed to investigate the genotoxicity of forty-three
 4178 industrial chemicals, methanol was assessed for its mutagenic potential in the Ames test with the
 4179 *Salmonella typhimurium* tester strains TA1535, TA1537, TA1538, TA98, TA100 and *Escherichia coli*
 4180 WP2 uvr A, using the preincubation method, both in the absence and presence of rat liver S9
 4181 metabolism. Dose-levels of 5, 10, 50, 100, 500, 1000, 5000 µg/plate were used. The Panel considered
 4182 that the methods implemented were sufficiently robust to support the results reported. Methanol was
 4183 not mutagenic in this test system either in the absence or in the presence of S9 metabolic activation
 4184 system.

4185 In the study by Lasne *et al.* (1984) which aimed to compare the sensitivity of the SCEs and *in vitro*
 4186 micronucleus test assays to detect cytogenetic effects induced by different chemical agents, methanol
 4187 was assessed for its potential clastogenicity for the induction of micronuclei in Chinese hamster V-79
 4188 cells at a single dose-level of 50 µl/mL for 48 hours in the absence of S9 metabolism only. Results
 4189 obtained indicate that methanol did not induce micronuclei, under the reported experimental condition.
 4190 However, the Panel noted that the treatment time used was exceedingly long compared to the 1.5-2
 4191 cell cycle length recommended by the OECD guideline no. 487 for the testing of chemicals.
 4192 Furthermore, the dose-level administered was 10 times greater than the maximum recommended dose-
 4193 level to be used in *in vitro* studies (e.g. 5 µl/mL) to keep culture physiological conditions. On these
 4194 grounds, the Panel considered that the methods implemented were not sufficiently robust to support
 4195 the results reported.

4196 *In vivo studies*

4197 Pregnant CD-mice were administered by gavage twice daily with 2500 mg methanol/kg bw on GD 6-
 4198 GD 10 (Fu *et al.*, 1996). No evidence of methanol-induced formation of micronuclei in the blood of
 4199 fetuses or mothers was found. However, the Panel noted that blood samples were collected eight days
 4200 after last administration of the compound. Normal procedures indicate sampling not later than 72
 4201 hours, Therefore this study was not taken into further consideration (Annex H).

4202 NEDO (1987) carried out an *in vivo* micronucleus test in 6 male SPF mice/group who received a
 4203 single gavage dose of 1050, 2110, 4210, and 8410 mg methanol/kg bw. Twenty-four hours later, 1000
 4204 cells were counted for micronuclei in bone marrow smears. The Panel considered that the methods
 4205 implemented were sufficiently robust to support the results reported. At any dose level, methanol did
 4206 not induce micronuclei significantly compared with the vehicle control (i.e. sterilized distilled water).

4207 In a recent study, DNA adducts were measured in several organs after oral administration to rats by
 4208 gavage of the stable isotope methanol (¹³CD₄-methanol) in doses of 500 mg/kg bw and 2000 mg/kg
 4209 bw per day for 5 days (Lu *et al.*, 2012). The method can distinguish between adducts formed by
 4210 endogenous substances and exogenous agents. It has already been used to study formaldehyde adducts
 4211 in the nose and at distant sites after inhalation exposure to rats (Lu *et al.*, 2010; Lu *et al.*, 2011) and to
 4212 cynomolgus macaques (Moeller *et al.*, 2011). The data of the study (Lu *et al.*, 2012) show that labelled
 4213 formaldehyde arising from [¹³CD₄]-methanol induced N2-hydroxymethyl-dG DNA adducts in
 4214 increasing numbers with increasing dose in all tissues, including liver, but in particular in the bone
 4215 marrow. The number of exogenous DNA adducts was lower than the number of endogenous
 4216 hydroxymethyl-dG adducts in all tissues of rats. The ratio of exogenous/endogenous dG adducts was
 4217 0.18 and 0.45 in bone marrow and 0.056 and 0.2 in the liver after doses of 500 mg/kg bw and 2000
 4218 mg/kg bw of stable isotope methanol respectively after adjusting for isotopic effects in the metabolism
 4219 of [¹³CD₄]-methanol.

4220 The Panel identified some concerns about the lower number of exogenous hydroxymethyl DNA
 4221 adducts measured in the analysed tissues of rats following administration of [¹³CD₄]-methanol at 500
 4222 mg/kg bw for 5 days compared to the number of endogenous hydroxymethyl DNA adducts. A
 4223 previous study has shown a slower metabolic conversion of deuterium-labelled methanol compared to
 4224 the unlabelled one (Brooks and Shore, 1971; Kraus and Simon, 1975). The Panel noted that the
 4225 isotope effect factor employed was the mean of several widely varied published values. Furthermore,

4226 the measurements of N-hydroxymethyl-dG and dA adducts was not direct but required an *in vitro*
 4227 reduction process with NaCNBH₃ to the corresponding N-methyl derivative. Based on the authors'
 4228 comments, this procedure appeared not to be quantitative as the rate of reduction was in the range of
 4229 65-85%. Furthermore, deuterium could have been exchanged by the hydride during the reduction
 4230 steps, which would have allowed for depletion of the labelled material and therefore led to an
 4231 underestimation of the number of exogenous DNA adducts. The Panel noted that the measurements of
 4232 endogenous N-hydroxymethyl-dA adducts in the untreated animal group and in methanol treated
 4233 group at 500 mg/kg bw for 5 days were rather variable especially in the case of white blood cells, bone
 4234 marrow and brain cells.

4235 Furthermore, the Panel noted that adduct formation is a biomarker of exposure of organs and tissues to
 4236 methanol and that a second step is necessary between DNA adduct formation and mutagenic events
 4237 (Swenberg *et al.*, 2008; Jarabek *et al.*, 2009). Moreover, the Panel noted that doses applied in the study
 4238 were in the lethal range for humans. Therefore, the Panel considered that the methods implemented
 4239 were not sufficiently robust to support the results reported, and that no conclusion could be drawn
 4240 from the study.

4241 No increase in sex-linked lethal mutations in either the wild type or the Basc strain of *Drosophila*
 4242 *melanogaster* was found after methanol feeding (1000 mM) (Gocke *et al.*, 1981).

4243 Pereira *et al.* (1982) reported that '*the oral administration in mice of methanol (1000 mg/kg),*
 4244 *increased the incidence of chromosomal aberrations particularly aneuploidy and exchanges and the*
 4245 *micronuclei of polychromatic erythrocytes*'. However, the study was available as an abstract only, and
 4246 as such, the results could not be validated.

4247 Ward *et al.* (1983) reported an increase in chromosome aberrations [exchanges (Robertsonian
 4248 translocation), aneuploidy] in male B6C3F1 mice upon a single dose of 1000 mg methanol/kg bw).
 4249 However, the Panel noted that the concurrent vehicle control showed 10 exchanges out 665 cells
 4250 scored. Since chromatid and chromosome exchanges are not expected in healthy animals from this
 4251 strain the Panel considered that the methods implemented were not sufficiently robust to support the
 4252 results reported.

4253 *In vivo studies by other routes of exposure (inhalation and intraperitoneal administration)*

4254 Although the Panel noted that inhalation studies and intraperitoneal administration provide routes of
 4255 exposure that are different from oral exposure, once methanol is absorbed, it is rapidly distributed to
 4256 all organs and the tissue/blood concentration ratios should be similar (Cruzan, 2009). Therefore,
 4257 similar effects from all three routes of exposure can be expected. Consequently, four *in vivo* reports
 4258 using inhalation or the intraperitoneal route of administration (Gocke *et al.*, 1981; Campbell *et al.*,
 4259 1991; McCallum *et al.*, 2011a; 2011b) were considered by the Panel as valid studies and useful for a
 4260 weight-of-evidence (WOE) approach in the assessment of the genotoxic potential of methanol.

4261 Gocke *et al.* (1981) examined the potency of methanol to induce micronuclei formation in bone
 4262 marrow upon intraperitoneal injection. Two male and two female NMRI mice were given 1920, 3200
 4263 or 4480 mg methanol/kg bw as single intraperitoneal injections. No significant increase in the number
 4264 of micronucleated polychromatic erythrocytes was observed. The Panel considered that the methods
 4265 implemented were sufficiently robust to support the results reported.

4266 In the study by Campbell *et al.* (1991), ten male C57BL/6J mice/group were exposed by inhalation to
 4267 0, 800, or 4000 ppm (0, 1048, and 5242 mg/m³) methanol, six hours/day, for five days. At sacrifice,
 4268 blood cells were examined for the formation of micronuclei. In addition, primary lung cells cultures
 4269 established following perfusion of lung with 0.13% trypsin, 0.01% EDTA and 50 U of collagenase/ml,
 4270 were examined for SCEs, CA and micronuclei. Furthermore, the synaptonemal complex damage in
 4271 meiotic prophase nuclei obtained from teasing seminiferous tubules and subsequent fixation of cells
 4272 was analysed. All endpoints studied gave negative results. However, the Panel noted that cytogenetic

4273 analyses in primary lung cultures are not validated procedures and, more specifically, the spontaneous
4274 incidences of micronuclei, SCEs and chromosomal aberrations were unexpectedly very high for
4275 primary cell cultures (23.2%, 11.2% and 6.9% respectively in experiment 1). Furthermore, the absence
4276 of a concurrent positive control strongly biased the negative outcome of the study. The Panel
4277 considered that the methods implemented were sufficiently robust to support the results reported.

4278 Groups of 10 male Swiss-Webster mice which had been kept on either a normal or a folate-deficient
4279 diet for 9 weeks before dosing, were given intraperitoneal methanol doses of 300, 600, 1200 or 2500
4280 mg/kg bw on 4 consecutive days. 24 hours after the last dose, the incidence of micronuclei in the
4281 RNA-positive erythrocytes of the animals was determined. The treatment with methanol did not
4282 significantly increase the incidence of micronuclei above the control values (O'Loughlin *et al.*, 1992).

4283 McCallum *et al.* (2011a; 2011b) investigated the potential indirect DNA-damaging activity of
4284 methanol by a reactive oxygen species-mediated mechanism. Male CD-1 mice (9-13 weeks old), New
4285 Zealand white rabbits (5 month old) and cynomolgus monkeys (3.4-5.7 years) received 2000 mg
4286 methanol/kg bw by intraperitoneal injection. Tissue oxidative DNA damage was assessed 6h post-
4287 dose, measured as 8-hydroxy-2'-deoxyguanosine (8-oxodG) by HPLC with electrochemical detection.
4288 The results obtained showed no methanol-dependent increases in 8-oxodG in bone marrow or spleen
4289 (McCallum *et al.*, 2011a) or lung, liver or kidney (McCallum *et al.*, 2011b) in any species. Chronic
4290 treatment of CD-1 male mice with 2000 mg/kg bw of methanol daily by intraperitoneal injection for
4291 15 days also did not increase 8-oxodG levels in bone marrow or spleen (McCallum *et al.*, 2011a) or
4292 lung, liver or kidney (McCallum *et al.*, 2011b). To further assess the possibility that the formation of
4293 8-oxodG could have been enhanced by methanol, but rapidly removed via excision repair, DNA
4294 repair-deficient mice lacking oxoguanine glycosylase 1 (Ogg1-deficient mice) were treated with
4295 methanol at 2000 mg/kg once by intraperitoneal injection and tissues and organs collected 6 and 24
4296 hours later and analysed for oxidative DNA damage. The results obtained indicated an accumulation
4297 of 8-oxodG levels in the untreated Ogg1-deficient mice compared to the wild type mice, but no
4298 evidence for methanol-induced oxidative DNA damage was observed in lung, liver or kidney despite
4299 increases in 8-oxodG levels by the positive control potassium bromate (McCallum *et al.*, 2011b) or in
4300 bone marrow or spleen (McCallum *et al.*, 2011a). In the study by McCallum *et al.* (2011b), the
4301 potential induction of 8-oxodG formation by methanol was further assessed in fibroblasts from Ogg1-
4302 deficient mice treated with methanol *in vitro*. The results obtained indicated an accumulation of 8-
4303 oxodG levels following a 3-hour treatment with the renal carcinogen potassium bromate (at 2.0 mM);
4304 however, the fibroblasts did not accumulate 8-oxodG following exposure to 125 mM methanol for 6
4305 hours. The Panel noted that in this *in vitro* assessment, methanol was administered at a dose-level far
4306 exceeding the recommended maximum dose-level of 10 mM to keep physiological treatment
4307 conditions (OECD test guidelines. 483 and 487) In addition, free radical-mediated hydroxynonenal-
4308 histidine protein adducts were reported not to be enhanced by methanol in primate bone marrow or
4309 spleen, or in rabbit bone marrow or mouse spleen, although modest increases were observed in rabbit
4310 spleen and mouse bone marrow. The authors concluded that taken together these observations suggest
4311 that methanol exposure did not promote the accumulation of oxidative DNA damage in lung, kidney
4312 or liver (McCallum *et al.*, 2011a) or in bone marrow and spleen (McCallum *et al.*, 2011b) in any
4313 species investigated including Ogg1-deficient mice. The Panel considered that the methods
4314 implemented were sufficiently robust to support the results reported and agreed with the conclusions
4315 of the authors.

4316 **5.2.3.1. Conclusion on the genotoxicity of methanol.**

4317 The *in vitro* genotoxicity data on methanol which were considered as relevant for the evaluation
4318 comprise a negative bacterial reverse mutation test, a negative gene mutation test with
4319 *Schizosaccharomyces pombe* and a negative prophage induction test with *Escherichia coli* WP2(λ).

4320 The *in vivo* genotoxicity data upon oral uptake on methanol considered as relevant for the evaluation
4321 comprise a negative mouse micronucleus test in bone marrow. In addition, in studies following intra

4322 peritoneal application or inhalation exposure of mice, rabbits or monkeys, no evidence of induction of
4323 DNA-damage (8-oxodG) formation, micronuclei and SCE was found.

4324 In a weight-of-evidence approach, the Panel also considered *in vivo* studies with inhalation and
4325 intraperitoneal applications. Taken together the Panel concluded that the data set is limited but that the
4326 available reliable *in vitro* and *in vivo* data did not indicate a genotoxic potential of methanol.

4327 Overall, the Panel concluded that available data do not indicate a genotoxic concern for methanol.

4328 Summary tables on the genotoxicity of methanol are presented in the Annex H.

4329 **5.2.4. Chronic toxicity and carcinogenicity of methanol**

4330 Methanol is a breakdown product of aspartame and was considered by the SCF in its safety assessment
4331 of aspartame (SCF 1985; 2002). The SCF concluded that there was no reason of concern regarding the
4332 chronic toxicity and carcinogenicity over the amounts of methanol likely to be produced by the
4333 metabolism of aspartame when compared with those present naturally in food (SCF, 1985). However,
4334 Soffritti and co-workers in their study recently proposed a role for methanol in the potential
4335 carcinogenic effects of aspartame (Soffritti *et al.*, 2010). Although the ANS Panel had previously
4336 concluded that the results of Soffritti did not provide evidence for a carcinogenic effect of aspartame
4337 in mice, the Panel decided to present a more detailed analysis of the suggested chronic toxicity and
4338 carcinogenicity of methanol (EFSA, 2011a). Only oral chronic and carcinogenicity studies of
4339 methanol have been considered for this evaluation.

4340 The oral studies on chronic toxicity and carcinogenicity of methanol are limited to a mouse study
4341 reported by Apaja (1980) and a rat study reported by Soffritti *et al.* (2002).

4342 The study by Apaja (1980) is part of a PhD thesis that investigated the effect of malonaldehyde bis-
4343 dimethylacetal that decomposes to malonaldehyde and methanol and aimed at studying the
4344 carcinogenic effect of malonaldehyde. The study design contained groups dosed with methanol but no
4345 concurrent unexposed controls. In the study, male and female Eppley Swiss Webster mice
4346 (25/sex/dose group; 8 weeks old at study initiation) were exposed 6 days per week until natural death
4347 to 0.222%, 0.444% and 0.889% methanol in drinking water (Apaja, 1980). Although no unexposed
4348 control group was included in the study, the author provided pathology data from historical records of
4349 untreated Swiss mice of the Eppley colony used in two separate chronic studies, one involving 100
4350 untreated males and 100 untreated females (Toth *et al.*, 1977) and the other involving 100 untreated
4351 females histopathological analyzed by Apaja (Apaja, 1980). Mice were housed five per plastic cage
4352 and fed Wayne Lab-Blox pelleted diet. Water was available *ad libitum* throughout life. Liquid
4353 consumption per animal was measured 3 times a week. The methanol doses in the drinking water
4354 study were reported as 22.6, 40.8 and 84.5 mg/day (560, 1000 and 2100 mg/kg bw/day) for females,
4355 and 24.6, 43.5 and 82.7 mg/day (550, 970, and 1800 mg/kg bw/day) for males, 6 days/week. The
4356 animals were checked daily and body weights were monitored weekly. Test animals were sacrificed
4357 and necropsied when moribund. The experiment ended at 120 weeks with the death of the last animal.

4358 The author reported that survival of the methanol-exposed females in the drinking water study was
4359 lower than that of untreated historical controls ($p < 0.05$), but no significant differences in survival was
4360 noted for males. An increase in liver parenchymal cell necrosis was reported in the male and female
4361 high-dose groups, with the incidence in females (8%) being significant ($p < 0.01$) relative to untreated
4362 historical controls. Incidence of acute pancreatitis was higher in high-dose males ($p < 0.001$), but did
4363 not appear to be dose-related in females, increasing at the mid- ($p < 0.0001$) and low-doses ($p < 0.01$)
4364 when compared to historical controls but not appearing at all in the high-dose females. Significant
4365 increases relative to untreated historical controls were noted in amyloidosis of the spleen, nephropathy
4366 and pneumonia, but the increases did not appear to be dose related.

4367 The author reported incidences of malignant lymphomas in females of 560, 1000, and 2100 mg/kg
4368 bw/day of 4/25 (16%), 9/25 (36%), and 10/25 (40%), respectively. Males from the drinking water

4369 study had incidences of malignant lymphoma of 1/25 (4%), 6/25 (24%), and 4/25 (16%) at 550, 970,
4370 and 1800 mg/kg bw/day. The lymphomas were classified according to Rappaport's classification
4371 (Rappaport, 1966), but it was not clear from the paper whether the lymphomas were generalised or
4372 whether they were restricted to one organ. The author indicated that the incidences in both males and
4373 females were 'within the normal range of occurrence of malignant lymphomas in Eppley Swiss mice'.
4374 However, elsewhere in the document, Apaja states that '*there was an increased ($p < 0.05$ in both)*
4375 *occurrence of malignant lymphomas (40.0% and 24.0% of the effective animals respectively)*
4376 *compared to historical data of untreated controls (Table 9)*'. The latter table reports the historical
4377 control values of Toth *et al.* (1977).

4378 The Panel noted the lack of a concurrent control group and the small number of animals used per dose
4379 group. In particular, the Panel noted that even though the incidence of malignant lymphomas in the
4380 high dose group appears higher than the historical data reported by Apaja (1980), a comparison with
4381 other contemporary control groups from the same institute and from the same colony of mice (see e.g.
4382 Cabral *et al.*, 1979) shows that the background lymphoma incidence in untreated animals can be as
4383 high as 26% for male and 43% for female mice. Furthermore, the study was inadequately described
4384 with respect to tumour nomenclature and description being poorly defined, and there was a lack of
4385 individual animal results regarding tumour incidence and time of death. Finally, the Panel noted that
4386 the animals were not maintained under pathogen-free conditions and this is reflected by an overall
4387 incidence rate of pneumonia of approximately 22% (Apaja, 1980). Overall, the Panel concluded that
4388 the study by Apaja is inadequate for the assessment of the carcinogenic potential of methanol.

4389 Soffritti *et al.* (2002) reported a chronic study in which methanol was given to 100 Sprague-Dawley
4390 rats/sex/group *ad libitum* in drinking water at concentrations of 0, 500, 5000, and 20000 ppm (v/v).
4391 The study was published as a non-peer reviewed paper. The animals were 8 weeks old at the onset of
4392 the study. All rats were exposed for up to 104 weeks, and after that maintained until they died
4393 naturally. The experiment ended at 153 weeks with the death of the last animal. Mean daily drinking
4394 water, feed consumption, and body weights were monitored weekly for the first 13 weeks, every 2
4395 weeks thereafter for 104 weeks, then every 8 weeks until the end of the experiment. Clinical signs
4396 were monitored 3 times/day, and the occurrence of gross changes was evaluated every 2 weeks. All
4397 rats were necropsied at death and underwent histopathological examination of organs and tissues.

4398 No substantial dose-related differences in survival were observed, although the data were not
4399 provided. Body weight and water and feed consumption were monitored in the study, but the data
4400 were not documented in the published report. However, average doses of 0, 55, 542 and 1840 mg/kg
4401 bw/day in males and 0, 67, 630 and 2250 mg/kg bw/day in females were calculated by Cruzan (2009)
4402 from drinking water concentrations of 0, 500, 5000, and 20000 ppm and data made public by the ERF.

4403 Water consumption in high-dose females was reduced compared to controls between 8 and 56 weeks
4404 and the mean body weight in high-dose males tended to be higher than that of control males. Overall,
4405 there was no pattern of compound-related clinical signs of toxicity, and the available data did not
4406 provide any indication that the control group was not concurrent with the treated group (Cruzan,
4407 2009). Soffritti *et al.* (2002) further reported that there were no compound-related signs of gross
4408 pathology or histopathological lesions indicative of non-cancer toxicological effects in response to
4409 methanol.

4410 The study reported a number of oncogenic responses to methanol including haemolymphoreticular
4411 neoplasms, the majority of which were reported to be lympho-immunoblastic lymphomas (Table 21).
4412 In the ERF bioassays, including this methanol study, haemolymphoreticular neoplasms are generally
4413 divided into specific histological types (lymphoblastic lymphoma, lymphoblastic leukaemia,
4414 lymphocytic lymphoma, lympho-immunoblastic lymphoma, myeloid leukaemia, histocytic sarcoma,
4415 and monocytic leukaemia) for identification purposes. According to Soffritti *et al.* (2007), the overall
4416 incidence of haemolymphoreticular tumours (lymphomas/leukaemias) in ERF studies is 13.3% (range,
4417 4.0–25.0%) in female historical controls (2274 rats) and 20.6% (range, 8.0–30.9%) in male historical
4418 controls (2265 rats). The high-dose responses, reported in the methanol study amounted to 28%

4419 (p<0.05) and 40% (not significant) for females and males, respectively. The study also suggested a
4420 significant increase in the incidence of ear duct carcinoma.

4421 In 2010, US Environmental Protection Agency (EPA) proposed that the chronic cancer study on
4422 methanol performed in rats by Soffritti *et al.* (2010) should be subjected to an independent pathology
4423 review following discrepancies in the diagnosis identified during a site visit to ERF by pathologists
4424 from NTP conducted in 2010. The EPA commissioned the NTP to conduct this pathology review. The
4425 NTP pathologists diagnosed fewer lymphoid neoplasms, mainly of the respiratory tract, and fewer
4426 neoplasms of the inner ear and cranium and noted that there was chronic inflammation of the nasal
4427 cavity, ear canal, trachea, and lung, indicating infection of the animals by one or more respiratory
4428 pathogens. EPA concluded that many of the malignant neoplasms and the lymphoid dysplasias
4429 diagnosed by the ERF pathologists were cases of hyperplasia related to chronic infection (Table 21,
4430 NTP-EPA, 2011).

4431 In view of the concerns identified by EPA, the Panel did not support the validity of the conclusions in
4432 studies reported by Soffritti and colleagues.

4433 **Table 21:** Summary Incidences of Malignant Lymphoma or Leukaemia in Male and Female Rats

	0 ppm ^c		500 ppm		5000 ppm		20000 ppm	
MALES								
Number of animals per group	100		100		100		98-100	
	ERF	PWG	ERF	PWG	ERF	PWG	ERF	PWG
Lung only	7	0	10	0	13	1	13	0
Multiple tissues	19	13	21	11	22	12	26	8
Total rats with Lymphoma ^a /Leukaemia ^b	26	13	31	11	35	13	39	8
FEMALES								
Number of animals per group	100		100		100		99-100	
	ERF	PWG	ERF	PWG	ERF	PWG	ERF	PWG
Lung only	3	3	5	0	6	0	7	0
Multiple tissues	9	5	16	4	16	7	18	6
Total rats with Lymphoma/Leukaemia	12	8	21	4	22	7	25	6
ERF = European Ramazzini Foundation study diagnosis, PWG = Pathology Working Group EPA Panel consensus								
^a ‘Lymphoma’ includes lymphoblastic lymphoma, lymphocytic lymphoma, lymphoimmunoblastic lymphoma for the ERF diagnoses. For QA pathologist and PWG, all lymphoma sub types were diagnosed as malignant lymphoma.								
^b ‘Leukaemia’ includes lymphoblastic leukaemia and myeloid leukaemia for SD diagnoses and for QA pathologist and PWG, includes myeloid leukaemia and mononuclear cell leukaemia.								
^c Average doses of 0, 55, 542 and 1840 mg/kg bw/day in males and 0, 67, 630 and 2250 mg/kg bw/day in females were calculated by Cruzan (2009) from drinking water concentrations of 0, 500, 5000, and 20000 ppm and data made public by the ERF.								

4434

4435 **5.2.5. Reproductive and developmental toxicity of methanol**

4436 A few studies on oral reproductive and developmental toxicity of methanol were reported. In most of
4437 the studies, especially in the rat, limitations were observed (maternal toxicity not described, limited
4438 number of animals and dose levels tested, time of dosing).

4439 Four studies were identified on the reproductive and developmental toxicity of methanol in mice via
4440 the oral route.

4441 **5.2.5.1. Reproductive toxicity**

4442 *Mice*

4443 Four month old male B₆C₃F₁ mice were dosed by gavage with 0 (n=5) or 1000 (n=10) mg/kg bw/day
4444 methanol in water for 5 days (Ward *et al.*, 1984). An increase in the number of mice with ‘banana-
4445 type’ sperm morphology was reported. The total number of sperm abnormalities was not significantly
4446 increased in the methanol group. The Panel noted that these effects were considered of unknown
4447 biological significance as fertility (by mating of the animals) was not tested and general toxicity was
4448 not described.

4449 **5.2.5.2. Developmental toxicity**

4450 *Mice*

4451 In a study by Rogers *et al.* (1993), twenty pregnant female CD-1 mice were administered with two
4452 daily doses by gavage of 2000 mg methanol/kg bw (total dose of 4000 mg methanol/kg bw/day) from
4453 GD 6-15; 8 control females received distilled water twice daily.

4454 At sacrifice at GD 18, there were four litters in the control groups and eight litters in the methanol
4455 group. Three females of the methanol group showed a fully resorbed litter and the number of live
4456 fetuses/litter was decreased. One female of the methanol group died. The percentage of fetuses per
4457 litter with cleft palate and exencephaly was 43.5 and 28.8, respectively in the methanol group. These
4458 abnormalities were not observed in the control group. The Panel considered the increase in totally
4459 resorbed litters and the decrease in the number of live fetuses/litter and the malformations observed to
4460 be compound-related.

4461 Both Fu *et al.* (1996) and Sakanashi *et al.* (1996) studied the influence of dietary folic acid on the
4462 developmental toxicity of methanol.

4463 In the study of Fu *et al.* (1996), virgin female CD-1 mice were assigned to diets containing either 400
4464 (marginal) or 1200 (control) nmol folic acid (FA)/kg, and 1% succinylsulfathiazole (to prevent
4465 endogenous synthesis of folate by the intestinal flora) for 5 weeks prior to mating and throughout
4466 breeding and gestation. From GD 6 -10 dams were given by gavage deionised, distilled water or
4467 methanol (MeOH, 15.65% Optima HPLC grade MeOH) at 2.5 g/kg body weight, twice daily. On GD
4468 18, mice were weighed and killed and the liver, kidneys, and gravid uteri removed and weighed (21-24
4469 litters/group). Implantation sites, live and dead fetuses, and resorptions were counted; fetuses were
4470 weighed individually and examined for cleft palate and exencephaly. The marginal FA dietary
4471 treatment resulted in low maternal liver (50% reduction) and red cell folate (30% reduction)
4472 concentrations, as well as low fetal tissue folate concentrations (60 to 70% reduction) relative to the
4473 adequate FA dietary groups. Marginal FA treatment alone resulted in cleft palate in 13% of the litters;
4474 there were no litters affected with cleft palate in the adequate FA-control group. Marginal FA-MeOH
4475 treatment resulted in a further increase in the litters affected by cleft palate (72% of litters affected).
4476 The percentage of litters affected by exencephaly was highest in the marginal FA-MeOH group. The
4477 authors considered that the results show that marginal folate deficiency in pregnant dams significantly
4478 increases the teratogenicity of MeOH.

4479 Sakanashi *et al.* (1996) fed CrI: CD-1 mice a purified, amino acid-based folic, acid-free diet fortified
4480 with either 400, 600, or 1200 nmol/kg diet folic acid commencing 5 weeks prior to mating and
4481 throughout breeding and gestation. All diets contained 1% succinylsulfathiazole to prevent
4482 endogenous synthesis of folate by intestinal flora. On GD 6-15, mice were gavaged twice daily with
4483 water or methanol [purity not specified] in water at 2000 or 2500 mg/kg bw for a total daily dose of 0,
4484 4000 or 5000 mg/kg bw. On GD 18, dams were weighed and killed and the liver, kidneys, and gravid

4485 uteri removed and weighed. Implantation sites, live and dead fetuses, and resorptions were counted;
4486 fetuses were weighed individually and examined for cleft palate and exencephaly. One third of the
4487 fetuses in each litter were examined for skeletal morphology. Twelve to 29 litters were examined per
4488 group; in the low folate/2000 mg methanol group only 3 litters were examined. Methanol treatment
4489 decreased gestational weight gain in groups fed diets containing 600 or 1200 nmol folic acid/kg diet;
4490 these effects were not seen in the 400 nmol/kg group. Methanol did not affect pregnancy or
4491 implantation rate. There was no consistent effect of methanol exposure on haematocrit or liver folate
4492 level; plasma folate was increased in mice from the 1200 nmol/kg group that received 5000 mg/kg/day
4493 methanol. Methanol decreased fetal body weight in each of the folic acid dietary groups. An increase
4494 in the litter incidence of cleft palate was seen with methanol treatment in all dietary groups; the
4495 incidence was exacerbated in the 400 nmol/kg group. The litter incidence of exencephaly was
4496 increased by exposure to methanol in the 400 nmol folic acid/kg group. Methanol increased anomalies
4497 affecting the cervical region, although the incidence tended to decrease in dietary groups receiving
4498 larger amounts of folic acid. The authors concluded that the developmental toxicity of methanol was
4499 enhanced when maternal folic acid stores were low.

4500 The Panel concluded from the two studies above, that the effects found on developmental toxicity
4501 were related to methanol. However, it is not proven that the enhancement of the developmental effect
4502 was due to the deficiency in folate as the effect described can also be related to malnutrition as also
4503 described in the NTP-CERHR 2003.

4504 *Rats*

4505 Three pre-natal developmental toxicity studies in rats were identified and are summarised below.

4506 Infurna and Weiss (1986) investigated in Long Evans rats the potential behavioural developmental
4507 toxicity following exposure of dams (GD 15 to 17 or GD 17 to 19) to a solution of 2% methanol
4508 (approximately 2500 mg/kg bw/day) in drinking water. The authors considered that the suckling
4509 behaviour and homing behaviour were affected in the methanol-exposed groups. No other
4510 reproductive or developmental effects or maternal toxicity was reported.

4511 Cummings (1993) dosed by gavage Holtzman rats (8/group) with water or 1600, 2400, or 3200 mg/kg
4512 bw/day methanol on GD 1 to 8. Maternal body weight was significantly decreased in the high-dose
4513 group on GD 9. On GD 20, no effects on litter size, fetal weight, resorptions or external abnormalities
4514 were observed. There were no effects on maternal ovary weight and corpora lutea. Methanol did not
4515 affect either uterine weight or maternal body weight on GD 20.

4516 Long-Evans rats (n=10-13/group) received by gavage a single dose of methanol of 1.3, 2.6, or 5.2
4517 ml/kg bw (corresponding to 1023, 2045, or 4090 mg/kg bw/day according to CERHR calculation) on
4518 GD10 (Youssef *et al.*, 1997). Signs of maternal toxicity (only at the highest dose group) included
4519 decreased body weight gain and feed intake. Fetal body weights were significantly reduced in all
4520 treatment groups, but not in a dose-dependent manner. Dose related-anomalies were undescended
4521 testes and eye defects, which reached the statistical significance in the high-dose group, and facial
4522 haemorrhage and dilated renal pelvises. The Panel considered this study to be of limited relevance due
4523 to the timing of the single dose, which was not at what is considered the most sensitive period for
4524 developmental toxicity of methanol.

4525 The Panel noted that the design of the rat studies was inadequate. In one study rats were only
4526 administered once and not during the most sensitive period for induction of developmental effects. In
4527 another study in rats the duration of the administration period (GD 1-8) did not cover the period of
4528 organogenesis (GD 6-15).

4529 **6. Discussion of methanol toxicity database**

4530 Methanol is subject to a significant first pass metabolism. Principally, methanol metabolism proceeds
4531 by stepwise oxidation via formaldehyde to formate, and then to carbon dioxide. Formaldehyde and

4532 formate can also enter the one carbon metabolic pool through tetrahydrofolic acid and from there, may
4533 contribute to the biosynthesis of purines, pyrimidines and amino acids. It is estimated that only 25% of
4534 ¹⁴C from ¹⁴C-methyl aspartame undergoes biosynthetic reactions based upon transmethylation through
4535 formylation (E92, 1976). The metabolism of formaldehyde to formate is very efficient with a half-life
4536 of approximately one minute (McMartin *et al.*, 1979; Tephly and McMartin, 1984). The oxidation of
4537 formate to carbon dioxide varies between species, the rate of formate elimination in humans and non-
4538 human primates being half of that in rats (Kavet and Nauss, 1990). In rodents, formate is converted to
4539 carbon dioxide through a folate-dependent enzyme system and a catalase-dependent pathway
4540 (Dikalova *et al.*, PNAS 2001) whereas in humans metabolism occurs exclusively through the folate-
4541 dependent pathway (Hanzlik *et al.*, 2005 DM&D).

4542 The adequate *in vitro* genotoxicity data on methanol comprised a negative bacterial reverse mutation
4543 test, a negative gene mutation test with *Schizosaccharomyces pombe* and a negative prophage
4544 induction test with *Escherichia coli* WP2(λ). The adequate *in vivo* genotoxicity data following oral
4545 administration of methanol comprised a negative mouse micronucleus test in bone marrow. In
4546 addition, in studies following intraperitoneal administration or inhalation exposure to mice, rabbits or
4547 monkeys, no evidence for induction of DNA-damage (8-oxodG), micronuclei and SCE was found.

4548 In a weight-of-evidence approach, the Panel concluded that the data set was limited but that the
4549 available reliable *in vitro* and *in vivo* data did not indicate a genotoxic concern for methanol.

4550 Soffritti *et al.* (2010) suggested that the metabolism of aspartame resulting in the formation of
4551 methanol might have played a role in the development of hepatocellular tumours. Therefore, the ANS
4552 Panel has undertaken a more detailed evaluation of the suggested role implied for methanol.

4553 The oral studies on chronic toxicity and carcinogenicity of methanol are limited to a mouse study
4554 reported by Apaja (1980) and a rat study performed by the ERF and reported by Soffritti *et al.* (2002).

4555 The Panel noted that the experimental design of the poorly reported study by Apaja (1980) is flawed
4556 due to the lack of a concurrent control group, the small number of animals used per dose group that
4557 were not maintained under pathogen-free conditions resulting in a high incidence rate of pneumonia.
4558 Therefore, the Panel concluded that the study by Apaja (1980) does not contribute to the assessment of
4559 the carcinogenic potential of methanol.

4560 The rat study on methanol (Soffritti *et al.*, 2002) demonstrated a statistically significant increased
4561 incidence of haemolymphoreticular tumours (lymphomas/leukaemias) but only in females at the
4562 highest dose level.

4563 As already discussed above, the validity of the ERF study on methanol has recently been severely
4564 criticised (Cruzan, 2009; Schoeb *et al.*, 2009; Schoeb and McConnell, 2011; NTP-EPA, 2011). The
4565 ANS Panel concurred with the concerns identified by EPA and others, and did not support the validity
4566 of the conclusions in the study on methanol as reported by Soffritti *et al.* (2002). The Panel concluded
4567 that the ERF rat study was not a suitable basis for the cancer risk assessment of methanol.

4568 No adequate animal studies on fertility and reproductive performance of methanol were described.

4569 In an oral pre-natal developmental toxicity study in mice on methanol by gavage from GD 6-15
4570 (Rogers *et al.*, 1993) only a control group and a high dose group (4000 mg/kg bw) were tested. The
4571 Panel concluded that an increase in totally resorbed litters and a decrease in the number of live fetuses
4572 per litter and an increased number of malformations were observed in the methanol treated group.

4573 In two other pre-natal developmental toxicity studies, the effect of a marginal folate level in the diet
4574 was tested (Fu *et al.*, 1996, Sakanashi *et al.*, 1996) in mice dosed with methanol by gavage from GD
4575 6-10 and GD 6-15, respectively. Fu *et al.* (1996) studied the effects of 4000 mg methanol/kg bw/day
4576 and Sakanashi *et al.* (1996) 4000 and 5000 mg/kg bw/day. At both dose levels, developmental effects

4577 of methanol were observed. However, the Panel concluded that it is not proven that the enhancement
4578 of the developmental effect was due to the deficiency of folate or if it was caused by malnutrition.

4579 In a rat pre-natal developmental toxicity study pregnant females were administered by gavage with a
4580 single dose on GD 10 of 0, 1023, 2045 or 4090 mg methanol/kg bw (Youssef *et al.*, 1997).
4581 Undescended testes and eye abnormalities reached statistical significance in the highest dose group.

4582 In another rat pre-natal developmental toxicity study, pregnant females were administered by gavage
4583 from GD 1-8 with 1600, 2400 or 3200 mg/kg bw/day (Cummings, 1993). No maternal or
4584 developmental effects were observed at sacrifice on GD 20.

4585 The Panel noted that the design of the rat studies was inadequate. In one study rats were only
4586 administered once and not during the most sensitive period for induction of developmental effects. In
4587 another study in rats the duration of the administration period (GD 1-8) did not cover the period of
4588 organogenesis (GD 6-15).

4589 The reproductive and developmental toxicity database of methanol is limited. Developmental effects
4590 were observed in mice, a species sensitive to developmental effects of methanol, after administration
4591 by gavage of 5000 mg/kg body weight/day from GD 6-10. In another study, developmental effects
4592 were observed after administration by gavage of 4000 and 5000 mg/kg body weight/day from GD 6-
4593 15. The Panel noted that there is 1000-fold difference between the dose at which effects were reported
4594 and the amount of methanol released from aspartame consumed at the ADI.

4595 The Panel noted that for average consumers of aspartame, the contribution to the overall exposure to
4596 methanol ranged from 1% up to 10% across the EU general population.
4597 In this estimate, the Panel also noted that exposure to methanol from natural sources is a minor
4598 contributing source compared to exposure from endogenous pathways (less than 10%).
4599 The Panel noted that the exposure from aspartame-derived methanol is similar to methanol exposure
4600 from natural sources.

4601 The Panel concluded that there is no safety concern from the levels of methanol released from
4602 aspartame under the current uses and permitted use levels.

4603 7. Biological and toxicological data on DKP

4604 A public call was launched by EFSA in 2011 and 2012. The present evaluation of the biological and
4605 toxicological data is based on the evaluation of unpublished and published studies following this call
4606 together with additional studies identified from the published literature up to the end of November
4607 2012.

4608 7.1. Absorption, distribution, metabolism and excretion of DKP

4609 7.1.1. Studies on absorption, distribution, metabolism and excretion of DKP received 4610 following a public call for data and which were available at the time of the previous 4611 JECFA and SCF evaluations

4612 The toxicokinetic properties of DKP have been studied extensively and are summarised below. Studies
4613 in the rat, rabbit, monkey and man (E15, E18, 1972) have shown that [¹⁴C(U)-Phe]-DKP (20-30 mg/
4614 kg bw) did not enter the normal metabolic pathways of phenylalanine and was not well absorbed from
4615 the gut. Radioactivity was recovered in the faeces, primarily as unchanged DKP. Following oral
4616 administration of DKP metabolism to phenylacetic acid occurs in the gastro intestinal tract,
4617 phenylacetic acid can then be absorbed and is rapidly excreted in the urine as phenylacetic acid and
4618 following conjugation with glutamate to phenylacetylglutamine (E80, 1974). The urinary excretion of
4619 phenylacetic acid and phenylacetylglutamine accounts for 20% (rat) to 50% (monkey and man) of the
4620 orally administered DKP. Phenylacetylglutamine is a naturally occurring compound that is also
4621 generated during the metabolism of phenylalanine. It was not observed following direct intravenous

4622 administration of DKP in monkeys, suggesting that it was generated by bacterial metabolism in the gut
4623 rather than by endogenous metabolism following absorption.

4624 When [¹⁴C(U)-Phe]-DKP was administered orally to monkeys (E80, 1974), the distribution curve was
4625 biphasic, with an early phase representing absorption and distribution among tissues while the second
4626 phase represented the appearance in the circulation of phenylacetylglutamine. Thus, the major
4627 metabolite of DKP, was the major form excreted via the urine in monkeys following oral
4628 administration of DKP. It has also been identified in human urine following administration of [¹⁴C(U)-
4629 Phe]-DKP (E80, 1974).

4630 Additional studies were carried out to characterise the biotransformation of DKP by intestinal flora
4631 (E80, 1974). The urinary metabolites of DKP were compared in conventional rats with normal
4632 microflora and in germ-free rats following dosing with [¹⁴C(U)-Phe]-DKP. In the absence of gut flora,
4633 89% of urinary ¹⁴C was present as DKP itself whereas in conventional rats only 24% of the urinary
4634 label was in this form with most of the urinary radioactivity recovered as phenylacetylglutamine.
4635 When the germ-free rats were populated with microflora, the pattern of metabolism was converted to
4636 that seen in conventional rats. It appeared, therefore, that the primary metabolites of DKP arose as a
4637 result of bacterial biotransformation.

4638 Cho *et al.* (1987) measured plasma and urinary concentrations of DKP in samples obtained from six
4639 normal adult subjects ingesting 2.2 mg DKP/kg bw as part of a dose of 200 mg aspartame/kg bw. DKP
4640 concentrations were measured by HPLC in plasma (0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 24 h)
4641 and urine (0-4, 4-8, 8-24 hours) for 24 hours. In plasma, DKP concentrations were always below the
4642 detection limit (less than 1 µg/ml). In urine, DKP was measurable in all collected samples. Mean total
4643 urinary DKP excreted during the first 24-hour period after dosing was 6.68 ± 1.30 mg (corresponding
4644 to 4.83 % of the ingested DKP dose). Approximately 44% of the total urinary excretion of DKP
4645 occurred in the first 4 hours after dosing.

4646 Another study reported the effect of repeated ingestion of beverages containing DKP on amino acid
4647 and DKP concentrations in plasma and urine of normal healthy subjects (UN05, 1987) and in PKU
4648 heterozygous subjects (UN06, 1987). Six normal healthy adult and six healthy adult PKU
4649 heterozygotes received a dose of 150 mg of DKP every hour for eight hours. Blood and urine samples
4650 were collected up to 24 hours. DKP administration produced a small increase in plasma DKP
4651 concentration that reached a plateau after four doses in both normal and PKU subjects. DKP was
4652 detectable in the urine during the 24-hour period following ingestion, and amounted to 5% or 2% of
4653 the total dose in normal or PKU heterozygous subjects, respectively. DKP had no significant effect on
4654 plasma amino acids concentrations, on blood formate and methanol, on urinary formate
4655 concentrations, on the plasma LNAA ratios in both normal and PKU subjects.

4656 7.2. Toxicological data of DKP

4657 7.2.1. Acute oral toxicity of DKP

4658 The acute toxicity of the DKP was studied in mice, rats and rabbits (E45, 1973). DKP was
4659 administered by gavage (as 15-17.5% suspension in 1% Tween 80, or an aqueous 2% suspension for
4660 low doses) at doses up to 5000 mg/kg bw. Male Sprague Dawley rats (n=6) were dosed at 2191 or
4661 5000 mg/kg bw and male Schmidt Ha/ICR mice (n=6) were dosed at 3710 or 5000 mg/kg bw. Male
4662 New Zealand White Luenberg rabbits were dosed at 2000, 2500 or 3200 mg/kg bw (n=1) and 4000 or
4663 5000 mg/kg bw (n=3). The animals were observed intermittently during the 7 day post-treatment
4664 period and no remarkable motor or behavioural activities were noted. No mortalities were observed in
4665 the experimental period.

4666 In the same report the acute toxicity of DKP was also studied by i.p. injection (E45, 1973). Male
4667 Sprague Dawley rats (n=6) were dosed at 1562 mg/kg bw and male Schmidt Ha/ICR mice at 1577

4668 mg/kg bw (n=6). Motor and behavioural activities were unremarkable and no deaths were recorded
4669 during the study period.

4670 As for aspartame, the authors concluded for DKP that 'it can be assumed that the LD50 values are in
4671 excess of the highest doses administered to each species by the routes employed'.

4672 The overview of these studies is presented in Annex F.

4673 7.2.2. Short-term and subchronic toxicity of DKP

4674 7.2.2.1. Studies on short-term and subchronic toxicity received following a public call for data 4675 which were available at the time of the previous JECFA and SCF evaluations

4676 DKP was administered daily for 2 weeks to Charles River CD rats by gavage (as 5% aqueous
4677 suspension) (E7, 1971). The daily dosage was 1000 mg/kg bw. The young adult rats (11 weeks) were
4678 randomly assigned, 5 males and 5 females to the control and treatment groups. Survival of the animals
4679 was 100%, no effects on body weight, or physical or behavioural changes were noted during the study
4680 period. In addition, no treatment-related changes in haematology, clinical chemistry, urinalysis or
4681 histopathology were reported.

4682 Groups of 10 Ha/ICR mice (4 weeks old; sex unspecified) were administered 1000 mg/kg bw DKP or
4683 an equal volume of vehicle only by gavage (as 5% aqueous suspension) daily for 2 weeks (E6, 1971).
4684 Survival of the animals was 100%, no effects on body weight, or physical or behavioural changes were
4685 noted during the study period, nor were any treatment-related changes in haematology, clinical
4686 chemistry, urinalysis or pathological changes reported.

4687 Groups of eight-week-old male and female Charles River CD rats (n=5) were randomly assigned to
4688 study groups (E8, 1972). DKP was administered in diet to result in dose levels of 0, 2000, 4000 and
4689 6000 mg/kg bw/day; actual consumption resulted in dosing within 5% of these doses. A moderate
4690 (statistically significant at sacrifice) decrease in body weight of the female rats in the highest dose
4691 group was noted and was attributed to a proportional decrease in feed intake. All animals survived the
4692 experimental period and no physical or behavioural changes were noted. There were no treatment-
4693 related changes in haematology, clinical chemistry, urinalysis or histopathology, but several
4694 incidences of non-treatment related disease were observed.

4695 The overview of these studies is presented in Annex G.

4696 7.2.3. Genotoxicity of DKP

4697 7.2.3.1. Studies on genotoxicity of DKP received following a public call for data which were 4698 available at the time of the previous JECFA and SCF evaluations

4699 *In vitro studies*

4700 DKP was tested in two bacterial reverse mutation assays with *Salmonella typhimurium* strains
4701 TA1535, TA1537, TA1538, TA98 and TA100, both plate incorporation without and with metabolic
4702 activation, (a) 10 to 5000 µg/plate (E98, 1978) and (b) 50 to 10000 µg/plate (E106, 1978) revealed no
4703 evidence for mutagenicity of DKP (Annex H). The Panel considered that the methods implemented
4704 were sufficiently robust to support the results reported.

4705 Negative results were also reported in a study in which DKP was tested in the Ames test with
4706 *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 at 870 and 4370 µg/ml
4707 both in the absence and presence of S9 and in limited test for reversion/mitotic crossing-over in the
4708 yeast D7, only without metabolic activation (Tateo *et al.*, 1989). The Panel noted that the study lacks
4709 TA 102 strain and that only two dose levels were tested.

4710 *In vivo studies*

4711 DKP was tested for the induction of chromosome aberrations in bone marrow cells in rats (10
4712 males/group) following administration of 0 (control), 250, 500, 1000 and 2000 mg DKP/kg bw/day by
4713 gavage for 5 consecutive days, given in three equally divided daily doses (E30, 1972). No evidence for
4714 CA-induction was found, the Panel considered that the methods implemented were sufficiently robust
4715 to support the results reported (Annex H)

4716 DKP was studied for mutagenicity by host-mediated assay in rats (E31, 1972). Rats were treated with
4717 0 (control), 250, 500, 1000, 2000 mg DKP/kg bw/day by gavage, divided equally in three doses for
4718 five consecutive days (E31, 1972). 30 minutes after the final dose, the animals were inoculated with
4719 *Salmonella typhimurium*, G-46, by intraperitoneal injection. Three hours later the bacteria were
4720 recovered and the peritoneal washing was evaluated for the presence of mutants. In rats, the host-
4721 mediated assay revealed no evidence for mutagenicity of DKP. However, the Panel noted that the test
4722 system employed has not received further validation.

4723 DKP was studied for mutagenicity by a host-mediated assay in mice (E82, 1974). Mice were treated
4724 orally with 0 (control), 1000, 2000, 4000 or 8000 mg DKP/kg bw/day, given in three equally divided
4725 doses at two-hour intervals for five consecutive days. The authors concluded that the study did not
4726 provide evidence for mutagenicity of DKP. However, for the reasons given in Annex H, the Panel
4727 considered that the methods implemented were not sufficiently robust to support the results reported.

4728 For a dominant lethal assay, rats were treated by gavage with 1000 mg DKP/kg bw/day, given in two
4729 equally divided doses on single day (E42, 1973). Each male was mated with sexually two mature
4730 virgin females weekly for eight consecutive weeks. On GD 14, these mated females were sacrificed
4731 for ovarian and uterine examinations. The panel considered that the methods implemented were
4732 sufficiently robust to support the results reported. DKP did not affect paternal growth, maternal
4733 pregnancy rate, corpora lutea and implantation sites.

4734 **7.2.3.2. Conclusion on the genotoxicity of DKP**

4735 The genotoxicity data on bacterial reverse mutation exhibit some limitations (e.g. absence of TA102
4736 and WP2 uvrA *Escherichia coli*) but were considered sufficient to conclude that DKP was not
4737 mutagenic in bacterial systems. The *in vivo* studies on genotoxicity of DKP comprise chromosome
4738 aberration in bone marrow erythrocytes and dominant lethal assays, all of which gave negative results.
4739 Taken together, the Panel considered that the data available *in vitro* and *in vivo* did not indicate a
4740 genotoxic potential of DKP.

4741 Overall, the Panel concluded that available data do not indicate a genotoxic concern for DKP.

4742 Summary tables on the genotoxicity of DKP are presented in the Annex H.

4743 **7.2.4. Chronic toxicity and carcinogenicity of DKP**4744 **7.2.4.1. Studies on chronic toxicity and carcinogenicity received following a public call for data
4745 which were available at the time of the previous JECFA and SCF evaluations**

4746 The chronic toxicity of DKP was investigated in mice (E76, 1974) and rat (E77-78, 1974).

4747 *Mice*

4748 DKP was administered in the diet to groups of 36 male and 36 female ICR Swiss albino mice at dose
4749 levels equating to 0 (control), 250, 500, and 1000 mg/kg bw/day for 110 weeks (E76, 1974). Another
4750 group of 72 male and 72 female served as controls. Criteria evaluated were physical appearance,
4751 behaviour, body weight gain, feed consumption, survival, clinical chemistry, eye examination, organ
4752 weights, tumour incidence and gross and microscopic pathology. Histopathological examination was

4753 performed on all gross lesions from all animals at each treatment level, and on 20-27 grossly
4754 unremarkable organs from control and high-dose level animals, as well as from roughly two-thirds and
4755 one-third of the animals in the mid- and low-dose groups. Detailed histopathological evaluations were
4756 performed on brain and urinary bladder from all mice in the control and the three treatment groups.
4757 There was no effect on the physical appearance and behaviour of the animals. Mean terminal body
4758 weights were significantly increased for only the low-dose males. No significant differences in body
4759 weight gain during the first year were noted despite significantly reduced feed consumption in males
4760 at all three doses of DKP. Mean survival was significantly decreased for the mid-dose females (16.7%
4761 \pm 6.3%) compared to the controls (33.9% \pm 5.7%). However, this finding was not considered
4762 treatment-related with respect to mean survival time. Mean survival to week 108 was 20-39%. No
4763 consistent treatment-related haematology/clinical chemistry and urinalysis findings were noted.
4764 Random fluctuations reaching statistical significance were occasionally observed but the values
4765 remained within historical control data. No compound-related effects in ocular examinations were
4766 reported. Gross observations at necropsy did not reveal compound-related changes in any organs or
4767 tissues. Relative organ weights were unaffected except for the relative thyroid weight that was
4768 increased in females at the mid- and high- doses. In no instance was the adjusted tumour incidence of
4769 any of the tumour types analysed significantly higher than that of the respective controls. No unusual
4770 tumours were encountered. One primary brain tumour (i.e. meningioma) was identified in a control
4771 female. Secondary lymphoreticular tumours were detected in brain and urinary bladder from small
4772 numbers of mice at all dose levels and controls. No consistent non-neoplastic alterations attributable to
4773 compound treatment were observed. The authors concluded that when DKP was administered to the
4774 mouse for 110 weeks in the diet at dose levels up to 1000 mg/kg bw/day there was no evidence of an
4775 effect with respect to the incidence of neoplasms or with regard to non-neoplastic changes in any
4776 organ or tissue. The Panel agreed with this evaluation and identified a NOAEL for this study of 1000
4777 mg/kg bw/day, the highest dose level tested.

4778 *Rats*

4779 DKP was administered in the diet to groups of 36 male and 36 female Charles River CD rats at dose
4780 levels of 0 (control), 750, 1500, 3000 mg/kg bw/day for 115 weeks (E77-78. 1974). Another group of
4781 72 male and 72 female served as controls. Histopathological examination was performed on available
4782 tissues from all animals in the study; also, all usual and unusual gross lesions and tissue masses were
4783 examined microscopically. Microscopic examinations of the brains, including seven coronal sections
4784 from each brain representing all major neuroanatomical areas, and bladder were also performed. There
4785 was no evidence that the administration of DKP at any level resulted in any effect on the physical
4786 appearance and behaviour of the animals. Two transient unidentified infectious diseases occurred
4787 during the study; these affected control and treated rats equally. One arose in weeks 12-14 of the study
4788 and was diagnosed as a putative viral infection of the salivary and Harderian glands. The other arose
4789 during weeks 48-52 and was assumed to be bacterial; rats were treated with penicillin G in an attempt
4790 to control this infection. No compound-related decrease in survival rates was observed in any of the
4791 group. Body weights were significantly reduced in the high-dose groups of both sexes in later stages,
4792 and periodically at lower doses. Feed intake was not consistently affected except for an increase in the
4793 mid-study in the high-dose male group. No statistically significant changes in haematology or clinical
4794 chemistry, and urinalysis were reported, except a mild but significant decrease in serum cholesterol in
4795 high-dose animals from day 42 (female) and 92 (male) onwards, and decreased urinary pH in all
4796 treated female groups, which was statistically significant at the high-dose level. This effect was
4797 thought to be associated with the presence of acidic DKP metabolites in urine. No compound-related
4798 effects in ocular examination were noted. No biologically meaningful compound-related variation in
4799 organ weights was observed. There was no treatment-related non-neoplastic histopathology in any
4800 organ or tissue, and no unusual non-neoplastic lesions were observed. The adjusted tumour incidence
4801 of any of the tumour types analysed was not significantly higher than that of the respective controls.
4802 No unusual tumours were encountered. The authors concluded that the dietary administration of DKP
4803 to rats for 115 weeks at dose levels up to 3000 mg/kg bw/day indicated no evidence of an effect with
4804 respect to the survival rate, rate of body weight gain, physical examination and haematology findings,
4805 and incidence of neoplasms or non-neoplastic changes in any organ or tissue. DKP produced a

4806 significant decrease in urinary pH in the high-dose female group, which was attributed to acidic
4807 metabolites of DKP in the urine, and a mild but significant decrease in serum cholesterol at the high-
4808 dose level only. In addition, a significant increase in uterine polyps (benign tumours) was also found
4809 in females treated with the mid- and high-doses of DKP (1/69, 1/34, 4/34, 6/33, in control, 750, 1500,
4810 3000 mg/kg bw/day, respectively). Based on these last observations, and in agreement with the JECFA
4811 monograph (JECFA, 1980) and the SCF evaluation (1989), the Panel identified a NOAEL for this
4812 study of 750 mg DKP/kg bw/day.

4813 **7.2.4.2. Additional chronic toxicity and carcinogenicity studies**

4814 An additional 2-year chronic toxicity and carcinogenicity study on aspartame was conducted in Wistar
4815 rats (Ishii *et al.*, 1981; Ishii, 1981). This study is described in section 3.2.4.1. This study also included
4816 a group of rats that was fed 4000 mg/kg bw/day aspartame + DKP (3:1) for up to 104 weeks. There
4817 was a dose-dependent depression of body weight gain at 4000 mg/kg aspartame + DKP (3:1) in males
4818 and females. This effect was correlated with decreased feed consumption. There was no evidence of a
4819 treatment-related effect on incidence of neoplastic and non-neoplastic changes other than an increase
4820 in focal mineralisation of the renal pelvis in both males and females (incidences in males: control,
4821 1/57; aspartame + DKP (3:1), 13/59; incidences in females: control, 16/59; aspartame + DKP (3:1),
4822 38/60) associated with a dose-related increase in urinary calcium (Ishii *et al.*, 1981; JECFA, 1981;
4823 EFSA, 2006). In addition, medullary mineralisation was observed in females (control, 23/59;
4824 aspartame + DKP (3:1), 36/60). The authors attributed these effects to irritation caused by the mineral
4825 deposition and considered to be of minimal toxicological significance. The Panel noted that
4826 mineralisation in the kidney is a common finding in rats, the aetiology being associated with mineral
4827 imbalance (Lord and Newberne, 1990). Thus, there was no evidence for toxicity of aspartame with its
4828 degradation product DKP over 2 years in rats. The Panel noted that this study provided information on
4829 the lack of toxicity of aspartame when administered in conjunction with DKP.

4830 **7.2.5. Reproductive and developmental toxicity of DKP**

4831 **7.2.5.1. Studies on reproductive and developmental toxicity received following a public call for** 4832 **data which were available at the time of the previous JECFA and SCF evaluations**

4833 *Rats*

4834 The available data were a segment I study (male and female fertility study; E37, 1973), a segment II
4835 study (embryotoxicity and teratogenicity; E38, 1973) and a segment III study (peri- and post-natal
4836 development; E13, 1972) in rats. In addition, there was a segment II study in rats given a mixture of
4837 aspartame and DKP (3:1) (E56, 1972). The route of administration was via the diet. Animals were fed
4838 the test material from 17 days prior mating through gestation and lactation (E37, 1973), from GD 6 to
4839 15 (E38, 1973), from GD 14 through weaning (E13, 1972), and from GD 6 to 14 (E56, 1972). None of
4840 the endpoints studied showed a difference between treated groups and controls and therefore, the
4841 lowest NOAEL observed in these studies was 2000 mg DKP/kg bw/day (E37, 1973; E38, 1973),
4842 corresponding to the highest dose tested. In the segment II study with a mixture of aspartame and DKP
4843 (E56, 1972), the NOAEL was 3040 mg/kg bw/day, the highest dose tested.

4844 *Rabbits*

4845 The rabbit studies included two developmental toxicity studies, which were confounded by poor health
4846 of the animals, and, in many cases, by a number of deaths in the treated groups, possibly related to
4847 misdosing due to gavage technique issues. In one study, DKP was administered to albino rabbits at
4848 doses of 500, 1000, and 2000 mg/kg bw/day from GD 6 to 18 (E57, 1972), whereas in a second study
4849 albino rabbits were administered a mixture of aspartame and DKP (3:1) at doses of 1000, 2000, and
4850 3000 mg/kg bw/day from GD 6 to 18 (E29, 1972). In the first study (E57, 1972), according to the
4851 authors the NOAEL for maternal toxicity was 1000 mg/kg bw/day, due to a large gastric bolus
4852 comprised of granular white material (presumed DKP) admixed with hair found at necropsy in some
4853 animals from the high-dose group. In the second study (E29, 1972), there was a statistically significant

4854 decrease in live mean fetal weights at the two highest doses, therefore according to the authors the
4855 NOAEL for this study was 1000 mg/kg bw/day (E29, 1972).

4856 **8. Discussion of DKP toxicity database**

4857 DKP is one of the degradation products of aspartame that is formed under certain processing and
4858 storage conditions. The EU and JECFA specifications for aspartame set a limit for DKP levels of 1.5%
4859 (w/w) in aspartame as supplied (Commission Regulation (EU) No 231/2012; JECFA, 2006). The
4860 levels of DKP will potentially increase in aspartame-containing food and drinks during processing and
4861 storage. For example, it has been determined that up to 24% of the aspartame is degraded into DKP in
4862 carbonate beverages (UN11, 1986; Table 3).

4863 The Panel noted that the content of DKP in aspartame tested in the toxicological studies, including the
4864 long-term studies and carcinogenicity studies ranged from 0.1% up to 4%.

4865 Some toxicological studies have been conducted on DKP.

4866 Studies in animals and man have shown that DKP was not well absorbed from the gut and was
4867 recovered in the faeces, primarily as unchanged DKP. Following oral administration of DKP
4868 metabolism to phenylacetic acid occurs in the gastrointestinal tract, phenylacetic acid can then be
4869 absorbed and is rapidly excreted in the urine as phenylacetic acid and following conjugation with
4870 glutamate to phenylacetylglutamine. The urinary excretion of phenylacetic acid and
4871 phenylacetylglutamine accounts for 20% (rat) to 50% (monkey and man) of the orally administered
4872 DKP. Phenylacetylglutamine is a naturally occurring compound that is also generated during the
4873 metabolism of phenylalanine.

4874 The available *in vitro* genotoxicity data on bacterial reverse mutation have some limitations (e.g.
4875 absence of assays with Salmonella strain TA102 and *Escherichia coli* strain WP2 uvrA) but were
4876 considered sufficient to conclude that DKP is not mutagenic in bacterial systems. The *in vivo* data on
4877 genotoxicity of DKP comprise chromosome aberration in bone marrow erythrocytes and dominant
4878 lethal assay, all of which gave negative results. Taken together, the Panel considered that the data
4879 available from *in vitro* and *in vivo* studies did not indicate a genotoxic concern for DKP.

4880 DKP administration to mice for 110 weeks in the diet at dose levels up to 1000 mg/kg bw/day
4881 indicated neither a carcinogenic effect nor a treatment-related increase in non-neoplastic lesions at the
4882 doses tested (E76, 1974). The Panel considered that the NOAEL was 1000 mg DKP/kg bw/day, the
4883 highest dose level tested.

4884 In another study, DKP was administered in the diet to male and female Charles River CD rats at dose
4885 levels of 0 (control), 750, 1500, 3000 mg/kg bw/day for 115 weeks (E77-78, 1974). A significant
4886 decrease at the high dose level in feed intake (males), body weight (both sexes), urinary pH (females)
4887 and a mild but significant decrease in serum cholesterol (both sexes) were reported. In addition, a
4888 significant increase in uterine polyps (benign tumours) was also found in females treated with the mid-
4889 and high-doses of DKP. Based on these last observations, and in agreement with the JECFA
4890 monograph (JECFA, 1980) and the SCF evaluation (1989), the Panel identified a NOAEL for this
4891 study of 750 mg DKP/kg bw/day.

4892 Several studies were performed in rats and rabbits to assess the reproductive and developmental
4893 toxicity of DKP. The lowest NOAEL observed in the rat studies was 2000 mg DKP/kg bw/day (E37
4894 and E38), the highest doses tested. For the rabbit studies, the NOAEL was 1000 mg/kg bw/day, for
4895 maternal toxicity and the effects observed on fetal weight.

4896 **9. Summary of biological and toxicological data on phenylalanine and aspartic acid**

4897 Both phenylalanine and aspartic acid occur naturally and are normally consumed as part of the diet.

4898 **9.1. Biological and toxicological data on phenylalanine**

4899 **9.1.1. Absorption, distribution, metabolism and excretion of phenylalanine**

4900 Phenylalanine exists as D and L enantiomers, and L-phenylalanine is an essential amino acid required
4901 for protein synthesis in humans (Salway, 2012). Dietary intake of L-phenylalanine along with
4902 endogenous recycling of amino acid stores are the major sources of L-phenylalanine in the body, while
4903 utilisation of L-phenylalanine occurs through integration into proteins, oxidation to tyrosine by
4904 phenylalanine hydroxylase (PAH) or, to a minor extent, conversion to other metabolites such as
4905 phenylpyruvate.

4906 PAH (EC 1.14.16.1) catalyses the stereospecific hydroxylation of L-phenylalanine to tyrosine in a
4907 reaction requiring tetrahydrobiopterin as a cofactor and molecular oxygen. PAH activity is mainly
4908 associated with the liver, although minor activity has been demonstrated in rat kidney (Richardson *et*
4909 *al.*, 1993). A five-fold greater rate of L-phenylalanine metabolism between rats and humans has been
4910 reported (Fernstrom, 1989). The human *PAH* gene is found on the long arm of chromosome 12 and
4911 contains 13 exons which encode a polypeptide of 452 amino acids. Mutations at the *PAH* locus result
4912 in phenylketonuria (PKU; MIM# 261600), an autosomal recessive inborn error of L-phenylalanine
4913 metabolism resulting in hyperphenylalaninaemia (HPA). Untreated PKU is associated with an
4914 abnormal phenotype including growth failure, microcephaly, seizures and intellectual impairment
4915 caused by the accumulation of L-phenylalanine and its by-products (see section 10).

4916 **9.1.2. Toxicological data on phenylalanine**

4917 **9.1.2.1. Studies in animals**

4918 Many studies have been conducted where rats and other experimental animals have been fed large
4919 amounts of L-phenylalanine in an effort to reproduce the biochemical and behavioural effects
4920 observed in PKU patients. The rats studies show a decrease in growth rate when fed large amounts of
4921 L-phenylalanine (reviewed in Harper, 1984; also Section 3.2.5.). Effects of oral administration of L-
4922 phenylalanine in pregnant rabbits are described in Section 3.2.5.

4923 Additional studies on the effect of oral administration of high levels of L-phenylalanine have been
4924 conducted in primates. In one study (Waisman and Harlow, 1965), experimental PKU was produced in
4925 six infant rhesus monkeys by feeding excessive quantities of L-phenylalanine (3000 mg/kg bw/day)
4926 soon after birth and for up to three years. The phenylketonuric monkeys had elevated plasma levels of
4927 phenylalanine, and excreted almost the same phenylalanine metabolites in the urine as humans. Grand-
4928 mal convulsions, observed in some children with phenylketonuria, were also observed in the
4929 experimental animals. The observed slowness in adapting to testing procedures, or even failure to
4930 adapt, and the inadequate performance was interpreted by the authors as an intellectual deficit induced
4931 by the excessive dose of L-phenylalanine. In another study conducted on nine rhesus monkeys (Gibbs
4932 and Smith, 1977), an intragastric preload of 1 g L-phenylalanine/kg bw, but not 1 g D-phenylalanine
4933 produced large reductions in food consumption. The authors proposed that L-phenylalanine releases a
4934 satiety signal.

4935 **9.1.2.2. Studies in humans**

4936 A large number of papers have investigated the untoward effects of uncontrolled diets in subjects
4937 suffering from PKU and have led to a substantial understanding of the toxic effects of L-phenylalanine
4938 intake in humans. Some of these are briefly summarised below.

4939 Studies have shown that infants with PKU are particularly vulnerable to adverse effects of L-
4940 phenylalanine if their diet is unrestricted (Smith, 1971; Giovannini *et al.*, 2012). High levels of blood
4941 phenylalanine during infancy and childhood can lead to severe, permanent brain damage, but the exact
4942 mechanism underlying the effects on neuronal function is not yet fully understood. During childhood,
4943 adolescence and adulthood tolerance to dietary L-phenylalanine may increase but is often very
4944 variable depending on the severity of the form of PKU. Infants born to mothers with PKU have also

4945 been found to be very susceptible to the toxic effects of L-phenylalanine presenting a high rate of birth
4946 defects and developmental disabilities: congenital heart disease, mental retardation, facial
4947 dysmorphism and intra uterine growth retardation.

4948 A detailed discussion on the effect of plasma phenylalanine level management strategies on health is
4949 found in Section 10.

4950 **9.2. Biological and toxicological data on aspartic acid**

4951 **9.2.1. Absorption, distribution, metabolism and excretion of aspartic acid**

4952 The metabolism of orally administered L-aspartic acid has been studied in several species and is
4953 summarised as follows (Oppermann, 1984). The majority of radioactivity following oral
4954 administration of [¹⁴C(U)]-aspartic acid to rats or monkeys is accounted to 60-70% by ¹⁴CO₂ expired
4955 in the air. This rapid and high level of elimination as CO₂ appears to result from both the
4956 decarboxylation of L-aspartate to alanine and CO₂ and the transamination reaction with pyruvate to
4957 form alanine and oxaloacetate in the intestinal mucosal cells. The alanine and oxaloacetate formed
4958 then enter the tricarboxylic acid cycle to form eventually CO₂. Approximately 25% of the [¹⁴C(U)]-
4959 aspartic acid dose is not accounted for in the expired air, urine or faeces but appears to be incorporated
4960 into normal body constituents or used for gluconeogenesis.

4961 **9.2.2. Toxicological data on aspartic acid**

4962 L-Aspartic acid is among the most common amino acids found in our diet. It is an excitatory
4963 neurotransmitter of the central nervous system (brain, spinal cord). In contrast to L-phenylalanine, L-
4964 aspartic acid is a nonessential amino acid.

4965 Excitatory amino acids are known to cause neurodegeneration when nerve cells expressing glutamate
4966 receptors become overstimulated following exposure to excessive levels of glutamate and L-aspartate
4967 (Rothman and Olney, 1995; Choi, 2001). The concentration of extracellular L-aspartate and glutamate
4968 in the central nervous system must therefore be controlled to prevent neuronal degeneration.

4969 Even though orally administered L-aspartate is rapidly metabolised and kinetic studies on aspartame
4970 have shown that plasma L-aspartate levels do not substantially change following bolus or repeated
4971 administration of aspartame (see Section 3.1), some authors (Olney, 1982; Reif-Lehrer, 1976) have
4972 speculated that aspartame-derived L-aspartate, especially when consumed with foods containing
4973 monosodium glutamate (MSG), would cause an increase in the combined plasma concentrations of L-
4974 aspartate and L-glutamate, which might pose a risk of focal neuronal degeneration.

4975 **9.2.2.1. Studies in Animals**

4976 Aspartate and glutamate can cause hypothalamic neuronal death in neonatal rodents, if given orally in
4977 large doses (500 mg/kg bw or higher) to infant animals (Olney, 1969; Olney and Ho, 1970; Lemkey-
4978 Johnson and Reynolds, 1974; Okaniwa *et al.*, 1979; Finkelstein *et al.*, 1983; Daabees *et al.*, 1985). For
4979 instance, in mouse pups, which may be considered the most sensitive of all animal species, single
4980 doses of 1000 mg L-aspartate/kg bw caused extensive hypothalamic neuronal necrosis. In another
4981 study, eight-day-old mice were administered by gavage aspartate at 0, 1.88, 3.76, 4.89, 5.64 and 7.52
4982 mmol/kg bw (corresponding to 0, 250, 500, 650, 750 and 1000 mg L-aspartate/kg bw) and the degree
4983 and extent of neuronal necrosis determined. Dose-dependent hypothalamic neuronal necrosis was
4984 found with 4.89 mmol L-aspartate/kg body wt and higher doses. The extent of neuronal necrosis was
4985 proportional to dose once a neurotoxic dose of L-aspartate was reached. The authors also reported that
4986 the highest concentration of L-aspartate in the plasma not inducing lesions was 870 ± 230 µM.

4987 Olney and co-workers (Olney and Sharpe, 1969; Olney *et al.*, 1972) also reported neuronal necrosis in
4988 neonatal nonhuman primates administered large bolus doses of glutamate (1000-4000 mg/kg bw
4989 subcutaneously or orally. This observation, however, could not be reproduced by a number of other
4990 scientists with either glutamate or aspartame at high dosages (reviewed by Butchko *et al.*, 2002).

4991 A 90-day aspartate feeding study (Tada *et al.*, 2008) performed in Fischer 344 rats (55 males and 55
4992 females) was recently evaluated by EFSA (EFSA, 2008). The animals were divided into 5 treatment
4993 groups and fed diets controlled for L-aspartic content. Dietary L-aspartic acid dosing was 0, 0.05,
4994 1.25, 2.5 and 5.0 %, corresponding to 0, 28.7, 715.2, 1470.2 and 2965.9 mg/kg bw/day for females and
4995 to 0, 26.9, 696.6, 1416.6 and 2770.2 mg/kg bw/day for males. No signs or symptoms of neurotoxicity
4996 were observed at any of the doses tested. A NOAEL of approximately 700 mg/kg bw/day for males
4997 was identified by the ANS Panel based on renal toxicity findings (an apparent dose-related
4998 regenerative renal tubules dilation in males accompanied by inflammatory cell infiltration) (EFSA,
4999 2008).

5000 **9.2.2.2. Studies in humans**

5001 Numerous intervention trials have been performed in adults with different aspartate compounds
5002 (sodium, magnesium, potassium-magnesium, buffered aspartic acid, arginine aspartate) in doses
5003 ranging from 1 to 10 g/day, for time periods between one single dose and four weeks. These studies
5004 have previously been reviewed by the ANS Panel (EFSA, 2008). None of these studies were
5005 undertaken to assess toxicity of aspartate intake, however, excluding reports on plasma amino acid
5006 imbalance and soft stools/diarrhoea, no other adverse effects were reported (Colombani *et al.*, 1999;
5007 Chouinard *et al.*, 1990).

5008 Consumption of 15 g of arginine aspartate daily, corresponding to 6.3 g L-aspartate, over 14 days in a
5009 double-blind, placebo-controlled cross-over trial by 14 healthy individuals resulted in significantly
5010 higher plasma levels of arginine, ornithine and urea as compared to placebo. In contrast, plasma levels
5011 of most other amino acids, including aspartic acid, and total amino acids were reduced (Colombani *et al.*,
5012 1999).

5013 **10. PKU data**

5014 PKU is an autosomal recessive disorder that leads to high levels of L-phenylalanine in the plasma and
5015 low level of tyrosine in the blood (Widaman, 2009; Zimmermann *et al.*, 2012). High plasma L-
5016 phenylalanine levels are neurotoxic, probably through its inhibitory action on the transport of other
5017 amino acids (leucine, isoleucine, valine, tyrosine, tryptophan, and lysine), necessary for the protein
5018 and neurotransmitter synthesis (e.g. serotonin) (Giovannini *et al.*, 2012). Furthermore, the inhibition of
5019 L-phenylalanine metabolism to tyrosine impacts on the formation of melanin and neurotransmitters
5020 derived from tyrosine such as dopamine, adrenaline, noradrenaline and thyroid hormones. HPA results
5021 due to a number of different mutations within the PAH allele producing a spectrum of phenotypes
5022 including classic phenylketonuria (PKU), moderate PKU, mild PKU and mild HPA (Zimmermann *et al.*,
5023 2012). For all these phenotypes, plasma L-phenylalanine levels are above 10 mg/dL (600 µM)
5024 when on an unrestricted diet.

5025 The restriction of dietary L-phenylalanine is the mainstay of PKU management. Patients with PKU
5026 have to avoid foods rich in protein (meat, fish, eggs, standard bread, dairy products, nuts, and seeds)
5027 and foods and drinks containing aspartame, flour, soya, beer or cream liqueurs. Therefore, PKU diets
5028 consist mainly of low-protein natural foods (vegetables, fruits and some cereals) that are modest in L-
5029 phenylalanine (Giovannini *et al.*, 2012). The aim of the dietary treatment is to avoid acute and chronic
5030 increased concentrations of L-phenylalanine in plasma and consequently in cerebral tissue, responsible
5031 for an important worsening of the neuronal performance and behaviour in PKU patients.

5032 Growth and brain development are most rapid during the first 6 months, and an infant with PKU is
5033 particularly vulnerable to adverse effects of L-phenylalanine if their diet is unrestricted (Smith, 1971;
5034 Giovannini *et al.*, 2012). High levels of plasma L-phenylalanine during infancy and childhood lead to
5035 severe, permanent brain damage, but the exact mechanism of the effects on neuronal function is not
5036 understood.

5037 According to the NIH Consensus Statement on phenylketonuria, it is assumed that levels below 10
5038 mg/dL (600 µM) do not lead to brain damage. Infants with PKU should start treatment to establish
5039 dietary control of their L-phenylalanine levels, ideally within 7–10 days of birth (NIH, 2000).

5040 Inversely, excessive restriction of L-phenylalanine intake during the first year of life is also
5041 detrimental to health given that L-phenylalanine is an essential amino acid that cannot be synthesised
5042 by the body. Cases of loss of weight, vomiting, listlessness, generalized eczematous rash and mental
5043 retardation that have been attributed to suboptimal L-phenylalanine intake.

5044 The reference values for plasma L-phenylalanine concentration recommended by NIH (NIH, 2000) are
5045 L-phenylalanine levels between 2–6 mg/dL (120-360 µmol/L) for neonates through 12 years of age,
5046 and L-phenylalanine levels between 2–10 mg/dL (120-600 µmol/L) after 12 years of age.

5047 Waisbren and co-workers (Waisbren *et al.*, 2007) assessed the reliability of plasma phenylalanine
5048 levels as a predictive biomarker of clinical outcomes in the development of treatments for PKU by
5049 performing a systematic literature review and meta-analysis of published trials of PKU, which
5050 included L-phenylalanine level and neurological and dietary compliance outcome measures. The
5051 authors found that each 100 µM increase in L-phenylalanine (assessed by mean Index of Dietary
5052 Control during the critical childhood period or lifetime through 18 years) predicted a 1.3- to 3.9-point
5053 decrease in IQ for early-treated patients with PKU, over a L-phenylalanine range from 394 to 750 µM.
5054 A correlation was also found between concurrent L-phenylalanine level and IQ for early-treated
5055 patients with PKU, in which each 100 µM increase in L-phenylalanine predicted a 0.5- to 1.4-point
5056 reduction in IQ, over a L-phenylalanine range from 429 to 1664 µM.

5057 Although the maintenance of a plasma L-phenylalanine concentration within the recommended range
5058 is clearly important in the treatment of PKU, a recent meta-analysis of 40 studies (Anastasoae *et al.*,
5059 2008) demonstrated that the stability of plasma L-phenylalanine levels may be more important to
5060 cognitive functioning (FSIQ, Full Scale Intelligence Quotient) in PKU children continuously treated
5061 from shortly after birth than the overall exposure to L-phenylalanine. The authors of the study
5062 concluded that occasional increases ('spikes') in plasma L-phenylalanine levels of approximately 600
5063 µM, which occur in the majority of children with PKU, may be benign, but that it is the long-term
5064 variability that appears to be important in the effects of L-phenylalanine on cognitive function
5065 (Anastasoae *et al.*, 2008). Cockburn and Clark (1996) suggested that adolescents and adults should
5066 continue the diet with the aim to maintain phenylalanine plasma concentration no higher than 700 µM.

5067 In women with PKU, the most critical phase in controlling the L-phenylalanine plasma concentration
5068 is pregnancy. In 1980, Lenke and Levy (1980) reported results of over 500 pregnancies of women with
5069 PKU who were not on low-phenylalanine diets during pregnancy. Infants born to mothers with PKU
5070 had a high rate of birth defects and developmental disabilities: congenital heart disease, mental
5071 retardation, facial dysmorphism and intra uterine growth retardation (IUGR). Although these infants
5072 would have carried one functional *PAH* allele from their fathers, the exposure of the fetus to high
5073 levels of phenylalanine from the mother's blood via the placenta appeared sufficient to cause pre-natal
5074 damage. These pre-natal-exposure effects show a dose-response relation, with higher levels of
5075 exposure leading to higher levels of disability.

5076 Data from the prospective international Maternal PKU Collaborative (MPKUC) Study (Koch *et al.*,
5077 2003), the French survey (Feillet *et al.*, 2004) and the United Kingdom PKU Registry (Lee *et al.*,
5078 2005) have shown that many features of the maternal PKU syndrome were preventable when dietary
5079 L-phenylalanine intake was restricted before conception or soon afterward (Maillot *et al.*, 2008).

5080 The MPKUC Study (Koch *et al.*, 2003) was initiated in 1984 to monitor pregnancies of women with
5081 PKU, to maintain mothers on a low L-phenylalanine diet throughout their pregnancies, and to study
5082 relations between levels of L-phenylalanine in the mothers' blood during pregnancy and birth and
5083 developmental outcomes in their offspring. Women with plasma L-phenylalanine levels >240 µM

5084 were enrolled; 382 women contributed to 572 pregnancies. The 413 offspring were examined at birth
5085 and annually. Congenital abnormalities were noted, and the focus was on the consequences of
5086 congenital heart disease and microcephaly on developmental outcome. To this effect, the McCarthy
5087 General Cognitive Index was administered at age four, followed by the Wechsler Intelligence Scale
5088 for Children (Revised) at age six. Microcephaly was noted in 137 offspring (33%), and 32 (7.7%) had
5089 congenital heart disease. Maternal plasma L-phenylalanine levels were higher for infants with
5090 congenital heart disease and microcephaly than for infants with congenital heart disease only ($P=0.02$).
5091 Mean L-phenylalanine levels at weeks four and eight of gestation predicted congenital heart disease
5092 ($P<0.0001$). The McCarthy General Cognitive Index score was lower with congenital heart disease
5093 ($P=0.005$) and microcephaly ($P=0.0017$), as was the Wechsler Intelligence Scale for Children
5094 (Revised) full-scale IQ (intelligence quotient) score ($P=0.0002$ for congenital heart disease and
5095 $P=0.0001$ for microcephaly). Optimal birth outcomes occurred when maternal plasma L-phenylalanine
5096 levels between 120 and 360 μM were achieved by 8 to 10 weeks of gestation and maintained
5097 throughout pregnancy.

5098 Widaman and Azen (2003) modelled the relationship between pre-natal exposure to L-phenylalanine
5099 and measures of offspring intellectual development in the offspring of the MPKUC Study (Koch *et al.*,
5100 2003). From the model, a nonlinear relation between pre-natal L-phenylalanine exposure and offspring
5101 cognitive outcomes, with damage to the developing fetus if average L-phenylalanine levels are above
5102 approximately 360 μM was derived. Moreover, pre-natal L-phenylalanine exposure had a strong effect
5103 on offspring outcomes at one year of age and was the only one of the background, pregnancy-related,
5104 or perinatal variables to directly influence offspring outcomes at 2, 4 and 7 years of age.

5105 Data from a retrospective review of pregnancies from January 1977 to June 2006 showed that an L-
5106 phenylalanine-restricted diet should start before conception to avoid malformations and congenital
5107 heart disease and to ensure the best possible developmental outcome for the offspring. The study
5108 concluded that not only was it important to aim to maintain maternal plasma L-phenylalanine
5109 concentrations of $<300 \mu\text{M}$, but, also, that variations in L-phenylalanine levels should be minimized
5110 (Maillot *et al.*, 2008).

5111 The Panel concluded that a critical threshold for the developmental and cognitive effects of L-
5112 phenylalanine is in the range from 330 to 360 μM , which is consistent with the NIH Consensus
5113 Statement on phenylketonuria (NIH, 2000). This document recommends that L-phenylalanine levels
5114 between 120 and 360 μM are achieved at least 3 months before conception and that metabolic control
5115 should be achieved as soon as possible. After 12 years of age, the range for recommended L-
5116 phenylalanine levels may increase to 120-600 μM . During pregnancy, the recommended level is 120-
5117 360 μM (NIH, 2000).

5118 **11. Mode of action**

5119 NOAELs of 4000 mg/kg bw/day identified in the chronic and carcinogenicity rat studies applying an
5120 uncertainty factor of 100, are understood to have served as the basis for the ADI of 40 mg/kg bw
5121 previously set by SCF and JECFA. Reanalysing the data the Panel noted that the effects reported in the
5122 rat and rabbit reproductive and developmental toxicity studies at doses below 4000 mg aspartame/kg
5123 bw/day should not be ignored, as the relationship of these effects to treatment could not be excluded.

5124 In the attempt to identify the causative agent, the Panel noted that aspartame is the methyl ester of the
5125 dipeptide of the amino acids, L-aspartic acid and L-phenylalanine. After oral ingestion, aspartame is
5126 hydrolysed, either within the lumen of the gastrointestinal (GI) tract, or within the mucosal cells lining
5127 the inside of the GI-tract. Hydrolysis is very efficient and the amount of aspartame that enters the
5128 bloodstream has been reported as undetectable in several studies conducted among others in rats, dogs,
5129 monkeys and humans (Oppermann, 1984; Burgert *et al.*, 1991). The products that result from the
5130 hydrolysis reaction are methanol and the amino acids aspartic acid and phenylalanine. Hydrolysis of
5131 aspartame releases equimolar quantities of aspartic acid and phenylalanine, together with methanol
5132 corresponding to 10% by weight of the aspartame dose. Further studies conducted in monkeys and

5133 pigs have shown that the potential intermediate metabolite phenylalanine methyl ester was rapidly
5134 broken down to phenylalanine and methanol in the intestinal lumen (Burton *et al.*, 1984; Burgert *et al.*,
5135 1991). The Panel concluded that hydrolysis of aspartame in the gastrointestinal tract was essentially
5136 complete and that there was no systemic exposure to aspartame but systemic exposure to aspartic acid,
5137 phenylalanine and methanol did occur.

5138 **11.1. Mode of Action Approach**

5139 Based upon their framework for assessing chemical carcinogens in experimental animals, the
5140 International Programme on Chemical Safety has published frameworks for assessing chemicals with
5141 cancer (Boobis *et al.*, 2006) and non-cancer endpoints (Boobis *et al.*, 2008). This type of approach
5142 enhances transparency and harmonisation of the risk assessment process.

5143 First, it was necessary to establish whether the weight-of-evidence from experimental observations
5144 was sufficient to ascertain a mode of action (MoA). Using the Bradford Hill criteria key events
5145 causally related to the toxic effect were identified. These key events were compared qualitatively and
5146 then quantitatively between experimental animals and humans. The decision tree from Boobis *et al.*
5147 (2008) was used to determine the human relevance of a MoA for toxicity observed in experimental
5148 animals. It must be noted that this Framework was an analytical tool that enabled a structured
5149 approach to assess the overall weight-of-evidence and its relevance to humans, it was not designed to
5150 provide an absolute answer on the sufficiency of the information available. The output from applying
5151 the Framework became the basis for continuation of the risk assessment.

5152 Application of the Framework was considered appropriate for evaluating the effects observed in
5153 reproductive and developmental toxicity studies after careful consideration of the weight-of-evidence
5154 for the observed toxicological responses following exposure to aspartame in experimental animals.
5155 The Panel chose to use this approach as the application of such a framework results in a transparent
5156 evaluation of the data; their application of the IPCS criteria are described in detail below.

5157 **11.1.1. Postulated Mode of Action for aspartame**

5158 The MoA proposed by the Panel for aspartame was that the toxicological effects observed in rat and
5159 rabbit in pregnancy was due to the metabolite phenylalanine.

5160 **11.1.2. Key events**

5161 The reproductive and developmental toxicity studies on aspartame consisted of an embryotoxicity and
5162 developmental study performed in the mouse, a two-generation study in the rat, five perinatal and
5163 postnatal developmental studies in the rat, a reproductive performance and developmental study in the
5164 rat and an embryotoxicity and teratogenicity study in the rat. In addition, eight embryotoxicity and
5165 teratogenicity studies were performed in the rabbit, four of which were by administration of aspartame
5166 in the diet and four by gavage. Some of these studies were also conducted with the aspartame
5167 metabolites phenylalanine and aspartic acid. In addition, studies were also available following
5168 administration of methanol.

5169 The Panel concluded that the depression in pup body weight in the high-dose groups could not be
5170 disregarded. The Panel noted that in earlier assessments of aspartame the observed effects were not
5171 considered in the evaluation. The published evaluations are not explicit on the rationale behind this
5172 decision. The Panel considered these effects might be due to high exposure to phenylalanine from
5173 metabolism of aspartame. This was supported by the findings in rats and rabbits treated with
5174 phenylalanine. The Panel further noted that although the data from study (E11, 1975) were sparse this
5175 study together with other segment I and segment III studies was considered sufficient to fulfil the
5176 requirements of a two-generation reproduction study in rats and derived from these studies a NOAEL
5177 of 2000 mg/kg bw/day based on the lower pup weights at weaning in both generations.

5178 The Panel agreed that the developmental rabbit studies may have been affected by the nutritional
5179 status as a result of gastrointestinal disturbances and the potential effect of phenylalanine. Overall, the

5180 Panel agreed that the rabbit studies should not be considered key because of the confounding factors
5181 affecting these studies and the likelihood that the gastrointestinal effects were species specific in
5182 rabbits without direct relevance to humans.

5183 **11.1.3. Biological Plausibility**

5184 Considering whether the MoA was consistent with what was known about the target process in general
5185 (biological plausibility), the Panel noted that there was long established evidence from human patients
5186 with phenylketonuria (PKU) that increased phenylalanine plasma levels during pregnancy result in
5187 adverse effects. The Panel noted that the database on PKU and particularly on effects during
5188 pregnancy had increased since the earlier evaluations.

5189 PKU is an autosomal recessive metabolic genetic disorder characterized by mutations in the gene for
5190 the hepatic enzyme phenylalanine hydroxylase (PAH), decreasing or inactivating its functionality.
5191 This enzyme is necessary to metabolize the amino acid phenylalanine to the amino acid tyrosine.
5192 When PAH activity is reduced, circulating phenylalanine levels are increased.

5193 Maternal PKU syndrome refers to the teratogenic effects of PKU during pregnancy. These effects
5194 include mental retardation, microcephaly, congenital heart disease, and intrauterine growth retardation.
5195 In untreated pregnancies wherein the mother has classic PKU with a plasma phenylalanine level
5196 greater than or equivalent to 1200 μM (20 mg/dl), abnormalities in offspring occur at exceedingly high
5197 frequencies, approximately 75-90% for microcephaly and mental retardation, and 15% for congenital
5198 heart disease. There is a dose-response relationship with progressively lower frequencies of these
5199 abnormalities at lower phenylalanine levels. The pathogenesis of this syndrome is unknown; it may be
5200 related to inhibition by phenylalanine of large neutral amino acid transport across the placenta or to
5201 direct toxicity of phenylalanine and/or a phenylalanine metabolite (phenylpyruvic acid) in certain fetal
5202 organs. Malformations and mental retardation in the offspring of women with PKU can be prevented
5203 by maintaining maternal plasma phenylalanine within a target range [120-360 $\mu\text{mol/L}$] through a
5204 phenylalanine-restricted diet.

5205 **11.1.4. Concordance of Dose-Response Relationships**

5206 Phenylalanine is released in a direct one to one molar ratio from the metabolism of aspartame, on a
5207 weight basis this would be 45% of the aspartame dose. The Panel noted that the effects observed in the
5208 reproductive and developmental toxicity studies in animals occurred at equivalent molar doses
5209 following administration of aspartame or phenylalanine.

5210 As described previously, PKU is an autosomal recessive disorder resulting in high levels of
5211 phenylalanine and low levels of tyrosine in the blood (Widaman, 2009; Zimmermann *et al.*, 2012).
5212 High blood phenylalanine levels are neurotoxic, mainly due to its inhibitory action on the transport of
5213 free L-amino acids (leucine, isoleucine, valine, tyrosine, tryptophan, and lysine), necessary for protein
5214 and neurotransmitter synthesis (dopamine and serotonin) (Giovannini *et al.*, 2012). It is recognised
5215 that malformations and mental retardation in the offspring of women with PKU can be prevented by
5216 maintaining maternal plasma phenylalanine within a target range 120-300 $\mu\text{mol/L}$ (or below a
5217 threshold thought to be between 330 and 360 $\mu\text{mol/L}$) through a phenylalanine-restricted diet.

5218 The form of the relation between pre-natal phenylalanine exposure and offspring cognitive outcomes
5219 appears to be nonlinear, with no damage to the developing fetus until exposure passes a critical
5220 threshold level. The best estimate of the critical threshold of phenylalanine exposure without damage
5221 to the offspring occurs is 330 to 360 $\mu\text{mol/L}$. A critical threshold in the range from 330 to 360 $\mu\text{mol/L}$
5222 is consistent with reference values indicated by the NIH Consensus Statement on phenylketonuria
5223 (NIH, 2000). However, the data presented by Lenke and Levy (1980), showed elevated rates of mental
5224 retardation even in offspring with maternal phenylalanine levels between 180 and 600 $\mu\text{mol/L}$. The
5225 Panel noted that control of phenylalanine plasma levels in PKU patients by dietary restriction can be
5226 highly variable and occasional high exposures or prolonged exposures above the target range can

5227 occur which could be responsible for effects observed in PKU patients controlling levels by dietary
5228 restriction.

5229 **11.1.5. Temporal Association**

5230 The effects were seen post administration. However apart from the hydrolysis of aspartame in the
5231 gastrointestinal tract, resulting in the increased plasma phenylalanine levels that the Panel believed
5232 was associated with the effects observed in the reproductive and developmental studies in animals,
5233 there is currently no further information on a step by step mechanistic explanation. This mechanistic
5234 explanation is lacking for animals and humans.

5235 **11.1.6. Strength, Consistency, and Specificity of Association of Toxicological Response with** 5236 **Key Events**

5237 There was consistency in the observed outcomes of the reproductive and developmental toxicity
5238 studies on aspartame in rat and rabbit; including a two-generation study in the rat, five perinatal and
5239 postnatal developmental studies in the rat, a reproductive performance and developmental study in the
5240 rat and an embryotoxicity and teratogenicity study in the rat and eight embryotoxicity and
5241 teratogenicity studies performed in the rabbit. Consistent observations from a number of studies with
5242 differences in experimental design, increases the support for the MoA. As the association was
5243 consistent, it was more likely that the relationship was causal. The effects observed in animals
5244 following aspartame exposure were reproduced if phenylalanine was given at the equivalent molar
5245 dose.

5246 **11.1.7. Other Modes of Action**

5247 Intact aspartame per se cannot be responsible for any adverse effects observed due to its complete
5248 hydrolysis within the GI tract.

5249 The Panel noted that the products formed following hydrolysis of aspartame were normal constituents
5250 of the diet (aspartic acid, phenylalanine and methanol) and were metabolised by endogenous metabolic
5251 pathways.

5252 Methanol is subject to a significant first pass metabolism. The overall metabolism of methanol
5253 proceeds by stepwise oxidation via formaldehyde to formate, and then to carbon dioxide.
5254 Formaldehyde and formate enter the one carbon metabolic pool through tetrahydrofolic acid and from
5255 there, may contribute to the biosynthesis of purines, pyrimidines and amino acids. The metabolism of
5256 formaldehyde to formate is very efficient with a half-life of about one minute (McMartin *et al.*, 1979;
5257 Tephly and McMartin, 1984). The oxidation of formate to carbon dioxide varies between species, the
5258 rate of formate elimination in humans and non-human primates being half of that in rats (Kavet and
5259 Nauss, 1990). In rodents, formate is converted to carbon dioxide through a folate-dependent enzyme
5260 system and a catalase-dependent pathway (Dikalova *et al.*, 2001) whereas in humans metabolism
5261 occurs exclusively through the folate-dependent pathway (Hanzlik *et al.*, 2005).

5262 The Panel noted that the methanol released from aspartame may enter the general circulation.
5263 However, on a weight basis the amount released would be one tenth of the aspartame dose.

5264 In studies on the reproductive and developmental toxicity of methanol effects were only observed at
5265 methanol doses greater than 2000 mg/kg bw. In studies on aspartame effects were seen at doses of
5266 1000-2000 mg/kg bw or higher. At these aspartame doses the maximum amount of methanol would be
5267 100-200 mg/kg bw respectively which is below the NOAELs for reproductive and developmental
5268 toxicity of methanol. The Panel therefore considered that the effects observed following aspartame
5269 could not be due to methanol.

5270 **11.1.8. Uncertainties, inconsistencies and Data Gaps**

5271 The Panel acknowledges that potentially the effects seen in rabbits may be secondary due to the fact
5272 the dams had a significant reduction in feed intake during pregnancy. The aborting rabbits from the
5273 high-dose group manifested body weight loss and lower to highly-restricted feed consumption,
5274 compared to those that maintained their pregnancies. However, the Panel was unable to determine
5275 whether the reported effects on the maternal organism and fetus were due to a reduced nutrient intake
5276 as suggested by the authors. The Panel noted that such a marked reduction in feed intake may have
5277 had a negative effect on the nutritional status of these females as a result of gastrointestinal
5278 disturbances and the potential effect of phenylalanine.

5279 The Panel further noted that the gastrointestinal effects in the rabbit may be species specific and would
5280 therefore not be relevant for humans. However, the Panel also considered a possibility that the high
5281 phenylalanine intake (from metabolism of aspartame) could at least in part be responsible for these
5282 effects in the high dose aspartame group because similar effects, although less severe, were seen in the
5283 phenylalanine dosed animals.

5284 In addition, there were no measures of plasma phenylalanine levels from the rat and rabbit
5285 reproductive studies. This constituted a data gap as the contemporaneous biochemical measures would
5286 increase confidence in the assumption that phenylalanine was responsible for the effects seen.

5287 Due to the limitations (in terms of dose and time points) in the kinetic data in animals, phenylalanine
5288 levels were not modelled. A further data gap acknowledged was the scarcity of the data on human
5289 plasma phenylalanine levels. From the data available following aspartame administration, it was only
5290 possible to model reliably the peak plasma phenylalanine levels, and estimates of other
5291 pharmacokinetic parameters were limited. Although potentially these could have been used to predict
5292 steady state concentrations of phenylalanine, had a more comprehensive dataset been available steady
5293 state phenylalanine levels could have been modelled more reliably and this would have been
5294 undertaken.

5295 **11.2. Assessment of postulated Mode of Action**

5296 Based on the metabolic data on the complete hydrolysis of aspartame in the gastrointestinal tract, the
5297 known effects of high phenylalanine concentrations on reproductive and developmental toxicity in
5298 several species and data demonstrating that equimolar concentrations of phenylalanine had similar
5299 effects on reproductive and developmental toxicity compared to aspartame which were not seen with
5300 either aspartic acid or methanol at the amounts produced from aspartame, the Panel considered that the
5301 proposed mode of action was credible and highly likely to explain the results seen in the animal
5302 studies with aspartame.

5303 **11.2.1. Life stage considerations**

5304 The key effects observed in rats and rabbit reproductive studies related to a specific life stage
5305 acknowledged to be critical in both species observed and to humans, thus the effects were considered
5306 as relevant for risk assessment. A spectrum of effects was observed in the experimental species,
5307 particularly abortion and growth restriction of the offspring. The latter effect was recognised as an
5308 important outcome in humans because it was associated with an increased risk of perinatal mortality
5309 and morbidity, including perinatal asphyxia (Barker, 2001). Moreover, there was epidemiological
5310 evidence that human fetal growth restriction correlates with adverse effects in adulthood. For example,
5311 affected individuals have an increased incidence of metabolic syndrome, manifesting as obesity,
5312 hypertension, hypercholesterolemia, cardiovascular disease, and type-2 diabetes (Barker, 1997).

5313 The Panel noted that neurodevelopment endpoints had not been measured in the animal studies,
5314 acknowledging that this would have provided highly relevant information on the mental retardation
5315 seen in PKU children.

5316 **11.2.2. Human relevance**

5317 A wealth of evidence exists from the human population (maternal PKU syndrome) showing that
5318 increased phenylalanine plasma levels during pregnancy result in adverse effects in the offspring,
5319 including mental retardation, microcephaly, congenital heart disease, and intrauterine growth
5320 retardation. It was highly plausible that the proposed mode of action for adverse effects observed in
5321 animals during pregnancy would be relevant to humans if sufficiently high aspartame exposures
5322 occurred.

5323 Lenke and Levy (1980) reported results from over 500 pregnancies of women with PKU who were not
5324 on low-phenylalanine diets during pregnancy. Infants born to mothers with maternal PKU syndrome
5325 had a high rate of birth defects and developmental disabilities: congenital heart disease, mental
5326 retardation, facial dysmorphism and fetal growth restriction.

5327 Data from the prospective international Maternal PKU Collaborative (MPKUC) Study (Koch *et al.*,
5328 2003), the French Survey (Feillet *et al.*, 2004), and the United Kingdom PKU Registry (Lee *et al.*,
5329 2005) showed that many features of the maternal PKU syndrome were preventable when dietary
5330 phenylalanine intake was restricted before conception or soon after conception (Maillot *et al.*, 2008).

5331 In contrast to these results are the findings of Teisser *et al.* (2012) who report data from 115
5332 pregnancies in 86 women. Ninety percent of women had been informed of the risk of maternal PKU in
5333 the absence of a strict diet during pregnancy, 88 % of women started a phenylalanine restricted diet
5334 before conception, and 45 % of infants were born small for gestational age (birth length and/or weight
5335 ≤ -2 SD). Phenylalanine intakes were lower in the group with fetal growth restriction from the fifth to
5336 the eighth month of pregnancy and duration of time spent at \pm phenylalanine levels ≤ 120 $\mu\text{mol/L}$
5337 during pregnancy was associated with a higher risk of fetal growth restriction. However, the Panel
5338 noted that as phenylalanine is an essential amino acid, it was likely that the increased risk of fetal
5339 growth restriction observed at low phenylalanine plasma levels in this study was due to dietary over
5340 restriction, i.e. providing not enough phenylalanine to allow normal development of the fetus.

5341 **11.2.3. Conclusion on the MoA analysis**

5342 The Panel considered that the proposed mode of action was plausible and relevant based on the
5343 weight-of-evidence available. There were uncertainties from the limited animal kinetic data and in the
5344 human dose response data. For the risk characterization of aspartame for humans, the Panel decided
5345 that the information on effects and dose response in PKU patients and human pharmacokinetic data
5346 were more appropriate than the results of animal studies of reproductive and developmental toxicity.

5347 **12. Dose-response modelling of plasma phenylalanine levels following aspartame**
5348 **administration**

5349 Data on the concentrations of phenylalanine in plasma after different doses of aspartame were
5350 extracted from various studies, mainly unpublished studies submitted in response to EFSA's call for
5351 data. These studies were principally conducted in the 1970's as part of the original evaluations of
5352 aspartame; the details of the studies are described in section 3.1. The studies involved a range of dose
5353 levels given as a single bolus administration. The time to reach peak plasma phenylalanine
5354 concentration (C_{max}) was comparable at all doses and varied from 30 minutes to 1 hour; thereafter
5355 levels fell sharply. The number of participants in each study differed, ranging from 6 to 20 (Annex J).

5356 Studies were conducted in three subsets of the population; individuals with 'normal' phenylalanine
5357 metabolism, heterozygous for phenylalanine hydroxylase, i.e. those with one recessive allele and those
5358 who are homozygous, i.e. individuals with PKU syndrome. The data (C_{max} values) from studies in
5359 normal and heterozygous individuals were used to generate a model that could be used in risk
5360 characterization. The data from PKU homozygotes were not used as these individuals are
5361 unrepresentative of the general population and specific labelling of aspartame containing products is
5362 required to permit these individuals to control dietary phenylalanine intake, which is a risk
5363 management issue.

5364 In specifying the modelling parameters, the Panel made various decisions. These decisions are
5365 described below together with the rationale for their choice, but were intended to reflect the ‘worst
5366 case’ scenario. Consequently, the model would be expected to incorporate an additional margin of
5367 safety.

5368 The first decision was to use data after a single bolus dose to represent the total daily aspartame intake,
5369 whilst acknowledging that normal consumption patterns would be in smaller quantities spread
5370 throughout the day. The administration of a bolus dose will overestimate the peak plasma
5371 concentration resulting from a daily dietary intake at the corresponding dose level. All the
5372 administration was as bolus doses which ranged from 4 to 200 mg/kg bw. This range included the
5373 current ADI of 40 mg/kg bw/day.

5374 The second decision was to use the measured peak plasma levels (C_{max} levels) following a single
5375 bolus administration as a surrogate for the response at the administered dose. Phenylalanine has a short
5376 half-life in plasma of approximately 1.7h (Filer and Stegink, 1991). The peak plasma levels were
5377 transient, and the Panel noted that these would be higher than steady state plasma levels arising from
5378 repeated intake at normal pattern of dietary intake of the same total daily dose .

5379 The third decision was to base comparisons on the mean plasma levels, which were assumed clinically
5380 safe for the critical endpoint (effects during pregnancy) in PKU patients. This subgroup of the
5381 population build up higher phenylalanine plasma levels which makes them more susceptible than the
5382 normal and heterozygous subgroups to adverse effects from phenylalanine during pregnancy. The
5383 plasma levels upon which the clinical advice is based are regarded as a threshold but the true threshold
5384 for adverse effects from phenylalanine would be expected to be higher. It was acknowledged by the
5385 Panel that it is not known whether effects seen at levels above the clinical target threshold were
5386 associated with the mean or peak plasma levels. The Panel noted that in PKU patients even with good
5387 dietary control of phenylalanine intake, plasma levels of phenylalanine can be highly variable and can
5388 result in either higher than targeted mean plasma levels or very high spike peak plasma levels. For
5389 PKU patients it is recognised that mild effects have been associated with mean plasma levels of 600-
5390 800 µM, whilst significant detrimental effects have been associated with both mean and spike peak
5391 plasma levels of 1100-1200 µM phenylalanine (Rouse *et al.*, 2000; Koch *et al.*, 2003).

5392 Current clinical practice guidelines recommend that PKU patients restrict their dietary intake of
5393 phenylalanine to keep plasma levels below 360 µM. The Panel noted that intakes of aspartame as a
5394 food additive could occur at the same time as other dietary phenylalanine sources. Therefore, the Panel
5395 considered that the threshold utilised for comparisons to the modelling should be lowered to allow for
5396 simultaneous intake of the food additive with meals. In toddlers it can be assumed that the daily
5397 exposure is taken up in five meals , in children in four meals, in adolescence, adults and the elderly in
5398 three meals rendering the phenylalanine intake per kg bw and meal into 14.4 to 27.3 mg/kg bw/meal
5399 (toddlers), 18.1-27.3 mg/kg bw/meal (children), 13.3 – 26.3 mg/kg bw/meal (adolescence), 11.8-19.6
5400 mg/kg bw/meal (adults), and 10.8 – 13.9 mg/kg bw/meal (elderly). The highest mean dietary
5401 phenylalanine exposure per meal is 27.3 mg/kg bw (Table 16) and this corresponds to a phenylalanine
5402 plasma concentration of 120 µM (as calculated from the dose-plasma phenylalanine modelling carried
5403 out and considering that phenylalanine represents 56 % by weight of aspartame) . Thus, the plasma
5404 phenylalanine from the diet (120 µM) has been subtracted from the current clinical guideline of 360
5405 µM to determine the maximum safe plasma concentration of phenylalanine that can be derived from
5406 aspartame (240 µM). The Panel noted that this plasma concentration was above normal phenylalanine
5407 plasma concentrations in man and therefore represents an overestimation of the phenylalanine intake
5408 from the regular diet (Levy, 2003).

5409 The Panel initially modelled the data for normal individuals, comparing dose with the highest peak
5410 plasma levels of phenylalanine in individuals to determine whether it was feasible to undertake dose-
5411 response modelling. Having considered the output from this initial model, the Panel considered that
5412 whilst modelling was feasible, a more sophisticated statistical model was appropriate. Subsequent
5413 modelling included multiple studies at some dose levels and allowed for the use of data from different

5414 studies and different numbers of subjects within the individual studies. The data were used in a model
5415 developed by the SAS Unit at EFSA and an internal report was provided to the Panel (Annex K). The
5416 model enabled an estimation to be determined of the plasma levels associated with the mean and 95th
5417 percentile confidence limits over the dose range studied.

5418 Based on the model, a plasma phenylalanine concentration of 240 µM would result from the
5419 administration of a bolus dose of 103 mg aspartame/kg bw (lower bound distributions: 88 mg
5420 aspartame/kg bw (CI 59-125) using a confidence level of 0.95) to a normal subject. For a heterozygous
5421 individual the concentration would be reached by the administration of a bolus dose of 59 mg
5422 aspartame/kg bw (lower bound distributions: 50 mg aspartame/kg bw (CI 28-69) using a confidence
5423 level of 0.95. The Panel considered that given the conservative assumptions, realistic dietary intake of
5424 aspartame and the confidence intervals provided by the modelling, the peak plasma phenylalanine
5425 levels would not exceed the clinical target threshold when an normal individual consumed aspartame
5426 at levels below the current ADI of 40 mg/kg bw/day.

5427 The Panel noted that studies on the effect of hourly ingestion of 600 mg aspartame over a period of
5428 eight hours by normal individuals have shown that plasma phenylalanine levels did not exceed the
5429 normal postprandial range (Stegink *et al.*, 1989). This dose corresponded to approximately 80 mg
5430 aspartame/kg bw or twice the current ADI, in one day. Moreover, using a published steady state model
5431 to compute the effect of repeated dosing of aspartame on plasma phenylalanine levels (Stegink *et al.*,
5432 1989), the Panel predicted that bolus administration of 40 mg/kg bw (the current ADI) on an hourly
5433 basis was required to achieve steady state plasma levels greater than 240 µM.

5434 The Panel therefore concluded that there would not be a risk of adverse effects on pregnancy in the
5435 general population including heterozygous individuals at the current ADI. The Panel noted that the use
5436 of a sensitive subpopulation (PKU patients) obviated the need for a toxicodynamic uncertainty factor
5437 (UF), for inter-individual variability. The Panel agreed that the use of these confidence levels in the
5438 dose-response modelling could replace the need for an additional toxicokinetic UF factor.

5439 **13. Risk characterisation**

5440 Based on the discussion of the aspartame toxicity database (Section 4) the Panel concluded that there
5441 were two key endpoints in the animal data for characterising the risk from aspartame consumption.
5442 These endpoints were: 1) reproductive and developmental toxicity; 2) chronic toxicity and
5443 carcinogenicity. The Panel concluded from the animal and human ADME data that aspartame was
5444 rapidly and completely hydrolysed in the GI tract to methanol and the amino acids phenylalanine and
5445 aspartic acid. The Panel concluded that any systemic effects following aspartame ingestion must be
5446 due to effects of these metabolites rather than aspartame per se.

5447 When considering all the genotoxicity, chronic toxicity and carcinogenicity studies on aspartame the
5448 Panel overall concluded that there was no convincing evidence for genotoxic or carcinogenic potential
5449 of aspartame in experimental animals. In the hazard identification and characterisation, the Panel has
5450 discussed the ERF studies performed in mice and rats and confirmed the conclusion of previous
5451 assessments that there were major deficiencies in these studies and as such, they were not suitable for
5452 hazard identification and characterisation. Based on the chronic toxicity and carcinogenicity data, the
5453 Panel concurred with the NOAEL of 4000 mg aspartame/kg bw previously identified by JECFA, the
5454 SCF and other assessors.

5455 In a Weight-of-Evidence approach, the Panel considered the database on the toxicity of methanol,
5456 including also *in vivo* studies by inhalation and intraperitoneal administration. Taken together the
5457 Panel concluded that the data set was limited but that the available reliable *in vitro* and *in vivo* data did
5458 not indicate a genotoxic potential of methanol.

5459 The oral studies on chronic toxicity and carcinogenicity of methanol were limited to a mouse study
5460 reported by Apaja (1980) and a rat study performed by the ERF and reported by Soffritti *et al.* (2002).

5464
5465 Overall, the Panel concluded that the study by Apaja did not contribute to the assessment of the
5466 carcinogenic potential of methanol.
5467
5468 As already discussed in section 4, the validity of the ERF study on methanol has recently been
5469 severely criticised (Cruzan, 2009; Schoeb *et al.*, 2009; Schoeb and McConnell, 2011; NTP-EPA,
5470 2011). The Panel concurred with the concerns identified by EPA and others, and did not support the
5471 validity of the conclusions in the study on methanol reported by Soffritti *et al.* (2002). The Panel
5472 concluded that the ERF rat study was not a suitable basis for the cancer risk assessment of methanol.
5473
5474 The reproductive and developmental toxicity database of methanol was limited. Developmental effects
5475 were observed in mice, a species sensitive to developmental effects of methanol, after administration
5476 by gavage of 5000 mg aspartame/kg body weight/day from GD 6-10. In another study, developmental
5477 effects were observed after administration by gavage of 4000 and 5000 mg/kg body weight/day from
5478 GD 6-15. The Panel noted that there was 1000-fold difference between the dose at which effects were
5479 reported and the amount of methanol released from aspartame consumed at the ADI.
5480
5481 Overall, the Panel concluded that the data on toxicity, kinetics, reproductive and developmental
5482 toxicity and genotoxicity of methanol do not suggest that there was a risk from methanol derived from
5483 aspartame at the current exposure estimates, or at the ADI of 40 mg/kg bw/day.
5484
5485 The Panel noted that aspartic acid is itself a neurotransmitter and can be converted to the more potent
5486 neurotransmitter glutamate. The Panel did not see any convincing evidence of neurotoxicity associated
5487 with aspartame. The Panel concluded that aspartic acid generated from aspartame was not of safety
5488 concern at the current exposure estimates, or at the ADI of 40 mg/kg bw/day.
5489
5490 The Panel considered that adverse effects reported for aspartame in animal studies could be attributed
5491 to the metabolite phenylalanine, which was particularly the case for the rat and rabbit developmental
5492 toxicity studies. The Panel noted that adverse developmental effects were seen in children born to
5493 PKU patients and that these effects appeared to be related to maternal phenylalanine levels. The Panel
5494 was aware that the knowledge on effects of phenylalanine in PKU mothers and their children both
5495 before and after birth had developed considerably since the initial evaluation of aspartame.
5496
5497 The Panel undertook a formal MOA analysis of the putative role of phenylalanine in the
5498 developmental toxicity seen in animal studies. This MOA analysis is described in Section 11.
5499
5500 The Panel considered that the proposed MOA was plausible and relevant based on the weight-of-
5501 evidence based on the available data summarised in the opinion. There were uncertainties from the
5502 limited kinetic data in animals and in the human aspartame dose-phenylalanine concentration response
5503 data. The Panel decided to base the risk characterisation on comparison of plasma phenylalanine levels
5504 following aspartame administration with plasma phenylalanine levels associated with developmental
5505 effects in children born from PKU mothers. The Panel decided these human data were more
5506 appropriate than the results of animal studies of reproductive and developmental toxicity for the risk
5507 characterization of aspartame.
5508
5509 Having established that the MOA was plausible and relevant, the Panel reviewed the information on
5510 plasma levels of phenylalanine associated with adverse effects on the fetuses of PKU mothers. The
5511 Panel noted that current clinical guidelines recommend that plasma levels of phenylalanine should be
5512 maintained below 360 µM. The Panel was aware that there continues to be discussion and research
5513 about the actual threshold and whether this was associated with mean or peak plasma concentrations
5514 of phenylalanine.
5515
5516 The Panel modelled peak plasma concentrations of phenylalanine following a bolus administration of
5517 aspartame to normal and PKU heterozygous individuals. The Panel chose assumptions, which
5518 overestimated the plasma concentrations of phenylalanine associated with administration of

5519 aspartame. These are discussed in more detail in Section 12. The Panel considered that comparing
5520 peak plasma concentrations derived in this way should be regarded as conservative/worst case
5521 scenario. In comparing these values to the plasma phenylalanine concentrations that have no effect on
5522 the fetuses of PKU mothers, the Panel took into account that aspartame could be consumed at the same
5523 time, as a meal that would contain proteins which when digested would release phenylalanine. In
5524 calculating a safe level of aspartame exposure (based on plasma phenylalanine concentrations), the
5525 Panel assumed the worst-case scenario that intake of aspartame occurs in combination with the meal
5526 which leads to circulating plasma phenylalanine concentrations of 120 μM (the maximum plasma
5527 concentration based on conservative assumptions of dietary exposure to phenylalanine). The
5528 concentration of plasma phenylalanine derived from aspartame was therefore set to 240 μM (i.e. 360
5529 μM minus 120 μM) by the Panel.

5530
5531 Based on the modelling, a plasma phenylalanine concentration of 240 μM would result from the
5532 administration of a bolus dose of 103 mg aspartame/kg bw (lower bound distributions: 88 mg
5533 aspartame/kg bw, 95th percentile, CI 59-125) to a normal subject. For a PKU heterozygous individual
5534 the concentration of 240 μM would be reached by the administration of a bolus dose of 59 mg
5535 aspartame/kg bw (lower bound distributions: 50 mg aspartame/kg bw, 95th percentile, CI 28-69). The
5536 Panel considered that given the conservative assumptions and the confidence intervals provided by the
5537 modelling, for realistic dietary intake of aspartame, the peak plasma phenylalanine levels would not
5538 exceed 240 μM .

5539
5540 The Panel noted that in the normal population the 95th percentile confidence interval for the lower
5541 bound of the dose for resulting in a peak plasma level of 240 μM following a bolus administration of
5542 aspartame was greater than 40 mg/kg bw (the current ADI). In the PKU heterozygous population the
5543 95th percentile confidence interval for the lower bound of the dose for resulting in a peak plasma level
5544 of 240 μM following a bolus administration of aspartame was greater than 40 mg/kg bw (the current
5545 ADI) in 82% of the simulations. The Panel considered that following bolus administration of
5546 aspartame of 40 mg/kg bw (the current ADI) the PKU heterozygous population would not exceed the
5547 current clinical guideline of 360 μM for plasma phenylalanine.

5548
5549 The Panel also noted that in order to exceed the phenylalanine plasma concentration of 240 μM
5550 following repeated administration of aspartame in normal individuals, a bolus administration at 40
5551 mg/kg bw (equivalent to the current ADI) would need to be given every hour.

5552
5553 The Panel considered the following:
5554 • the conservative assumptions used in the modelling, which would all overestimate peak
5555 plasma concentrations,

5556 • the available information on adverse effects in development in humans with PKU,

5557 • comparison with a concentration of 240 μM to allow for simultaneous ingestion of
5558 phenylalanine from other components of the diet in order to not exceed the current clinical
5559 guideline of 360 μM

5560 • results of the modelling

5561 • data from repeated oral administration of aspartame in humans

5562 • bolus intakes based on consumption of one litre of soft drink containing aspartame at the MPL
5563 of 600 mg/L by a child of 20-30 kg are unlikely to exceed 30 to 20 mg/kg bw, respectively.

5564 Based on these considerations and evaluations, the Panel concluded that under realistic conditions,
5565 phenylalanine plasma levels would not exceed 240 μM in normal or PKU heterozygous individuals.
5566 The Panel noted that this was considerably below the concentrations at which adverse effects in the

5567 fetus were reported and was also below the current clinical guideline (360 μ M) for prevention of
5568 effects in the fetuses of pregnant PKU patients. The Panel noted that in young children who did not
5569 suffer from PKU, plasma levels of phenylalanine resulting from aspartame ingestion at or below the
5570 ADI (as either a bolus or other aspartame consumption patterns) were likely to remain below 240 μ M.
5571 For pregnant women, the Panel noted that there was no risk to the fetus from phenylalanine derived
5572 from aspartame at the current ADI (40 mg/kg bw/day) in normal or PKU heterozygous individuals.
5573

5574 The Panel noted that it was currently not possible to include chronic endpoints in the postulated MOA.
5575 The Panel noted that the ADI previously derived by JECFA and the SCF of 40 mg/kg bw/day
5576 appeared to be based on the long-term animal studies using the default uncertainty factor of 100. The
5577 Panel considered that this remained appropriate for the evaluation of long-term effects of aspartame.
5578 The current evaluation, was based on analysis of human reproductive and developmental effects of
5579 phenylalanine in PKU patients, who are more susceptible than the general and PKU heterozygous
5580 population. Therefore, no additional allowance for toxicodynamic variability was required. The
5581 modelling of the aspartame dose- phenylalanine concentration response was based on data from PKU
5582 heterozygous individuals who at any dose would have a higher plasma phenylalanine concentration
5583 than the normal population, therefore no additional allowance for toxicokinetic variability was
5584 required. The Panel concluded that exposures at or below the current ADI were not of safety concern
5585 for reproductive and developmental toxicity in humans excluding PKU homozygous individuals.
5586

5587 The Panel concluded from the present assessment of aspartame that there were no safety concerns at
5588 the current ADI of 40 mg/kg bw/day. Therefore, there was no reason to revise the ADI for aspartame.
5589

5590 The Panel emphasised that its evaluation of phenylalanine plasma levels from a dose of aspartame at
5591 the ensuing ADI is not applicable to PKU patients. These individuals require total control of dietary
5592 phenylalanine intake to manage the risk from elevated phenylalanine plasma levels. The Panel noted
5593 that risk managers have recognised this by requiring appropriate labelling to indicate that aspartame is
5594 a source of phenylalanine.
5595

5596 Conservative estimates of exposure to aspartame made by the Panel for the general population were up
5597 to 36 mg/kg bw/day at the 95th percentile. These were below the ADI. The Panel noted that the
5598 percentage of PKU heterozygous individuals with lower bound below 36 mg would be considerably
5599 lower. Due to the conservatism of both the exposure assessment and the aspartame dose-phenylalanine
5600 concentration response modelling, the Panel considered that it was highly unlikely that any individual
5601 in the normal and PKU heterozygous population would have plasma levels of phenylalanine above
5602 240 μ M following aspartame ingestion. Based on the available data, the Panel further considered that
5603 even in combination with diet, these aspartame intakes (either as a single or repeated dose) would not
5604 lead to peak plasma phenylalanine concentrations above the current clinical guideline for prevention
5605 of adverse effects on the fetuses of PKU mothers of 360 μ M.
5606

5607 The Panel noted that the current ADI for DKP is 7.5 mg/kg bw/day. Based on the current specification
5608 for DKP in aspartame (1.5%), estimates of DKP exposure at the ADI for aspartame were below the
5609 ADI for DKP. Based on available data on DKP levels in food products following breakdown of
5610 aspartame (up to 24%), the Panel estimated high level exposure for the general population up to 5.5
5611 mg DKP/kg bw/day at the 95th percentile which is still below the ADI for DKP.
5612

5613 CONCLUSIONS

5614 The Panel concluded that chronic toxicity and reproductive and developmental toxicity were the
5615 critical endpoints in the animal database. The Panel considered that the evaluation of long-term effects
5616 of aspartame should continue to be based on the animal data. Based on a MOA analysis and the
5617 weight-of-evidence, the Panel considered that the reproductive and developmental toxicity in animals
5618 was due to phenylalanine released from aspartame and concluded that the basis for evaluation of the
5619 reproductive and developmental endpoint should be the available data in humans.

5620

5621 Conservative estimates of exposure to aspartame made by the Panel for the general population were up
5622 to 36 mg/kg bw/day at the 95th percentile.

5623 Due to the conservatism of both the exposure assessment and the aspartame dose-phenylalanine
5624 concentration response modelling, the Panel considered that it was highly unlikely that any individual
5625 in the normal and PKU heterozygous population would have plasma levels of phenylalanine above
5626 240 µM following aspartame. The Panel further considered that even in combination with diet, these
5627 aspartame intakes would not lead to peak plasma phenylalanine concentrations above the current
5628 clinical guideline for prevention of adverse effects on the fetuses of PKU mothers of 360 µM.
5629

5630 The Panel concluded from the present assessment of aspartame that there were no safety concerns at
5631 the current ADI of 40 mg/kg bw/day. Therefore, there was no reason to revise the ADI for aspartame.

5632 The Panel emphasised that its evaluation of phenylalanine plasma levels from a dose of aspartame at
5633 the ensuing ADI is not applicable to PKU patients. These individuals require total control of dietary
5634 phenylalanine intake to manage the risk from elevated phenylalanine plasma levels.
5635

5636 **DOCUMENTATION PROVIDED TO EFSA**

5637 1) List of unpublished study submitted to EFSA following the public call for data.

E1	Sc-18862: A Sweetening Agent	1972	Ajinomoto and Nutrasweet
E2	Sc-18862: Four Week Oral Tolerance Study In The Mouse	1972	Ajinomoto and Nutrasweet
E3	Sc-18862: Four Week Oral Tolerance Study In The Rat	1972	Ajinomoto and Nutrasweet
E4	Sc-18862: Nine Week Oral Toxicity Study In The Rat	Not reported	Ajinomoto and Nutrasweet
E5	Sc-18862: Evaluation Of Embryotoxic And Teratogenic Potential In The Rat	1970	Ajinomoto and Nutrasweet
E6	Sc-19192: Two Week Oral Toxicity Study In The Mouse	1971	Ajinomoto and Nutrasweet
E7	Sc-19192: Two Week Oral Toxicity Study In The Rat	1971	Ajinomoto and Nutrasweet
E8	Sc-19192: Five Week Oral Toxicity Study In The Rat	1972	Ajinomoto and Nutrasweet
E9	Sc-18862: Toxicological Evaluation In The Neonatal Rat (Final Report)	1972	Ajinomoto and Nutrasweet
E10	Toxicological Evaluation Of Sc-18862: Evaluation Of Reproductive Performance. Segment I Of The Teratology-Reproduction Profile	1972	Ajinomoto and Nutrasweet
E11	Sc-18862: Two Generation Reproduction Study - Rats (Final Report)	1971	Ajinomoto and Nutrasweet
E12	Sc-18862: Mutagenic Study In Rats (Final Report)	1970	Ajinomoto and Nutrasweet
E13	Sc-19192: Segment III - Perinatal Weaning Study In The Rat (Final Report)	1972	Ajinomoto and Nutrasweet
E14	Behavioral Effects Of Chronic Feeding Of L-Phenylalanine And Sc-18862 To Weanling Rats	1972	Ajinomoto and Nutrasweet
E15	The Metabolism Of Aspartame - Volume I Parts I-XIV	1972	Ajinomoto and Nutrasweet
E16	Sweetening Agents (Bibliography)	Not reported	Ajinomoto and Nutrasweet
E17	The Metabolism of Aspartame - Volume 2 Parts XV-XIX	1972	Ajinomoto and Nutrasweet

E18	The Metabolism of Aspartame - Volume 3 Parts XX-XXIII	1972	Ajinomoto and Nutrasweet
E19	Sc-18862: A Sweetening Agent (Endocrine Studies)	Not reported	Ajinomoto and Nutrasweet
E20	Sc-18862: 2 Month Oral Administration - Rats (Final Report)	1969	Ajinomoto and Nutrasweet
E21	Sc-18862: 2 Month Oral Toxicity - Dogs (Final Report)	1969	Ajinomoto and Nutrasweet
E22	Chicken Embryo Study Sc-18862 Calcium Cyclamate Sucrose (Final Report)		Ajinomoto and Nutrasweet
E23	Short Term Tolerance Of Aspartame By Normal Adults	1972	Ajinomoto and Nutrasweet
E24	Short Term Tolerance Of Aspartame By Obese Adults	1972	Ajinomoto and Nutrasweet
E25	Short Term Tolerance Of Aspartame By Adult Pku Heterozygotes	1972	Ajinomoto and Nutrasweet
E26	Tolerance Of Loading Doses Of Aspartame By Phenylketonuric (Pku) Homozygous Children	1972	Ajinomoto and Nutrasweet
E27	Sc-18862: 46 Week Oral Toxicity Study In The Hamster	1972	Ajinomoto and Nutrasweet
E28	Sc-18862: 106 Week Oral Toxicity Study In The Dog	1972	Ajinomoto and Nutrasweet
E29	Sc-18862 and 19192 (3:1 Ratio): Segment II Teratology Study In The Rabbit (Final Report)	1972	Ajinomoto and Nutrasweet
E30	Sc-19192: An Evaluation Of Mutagenic Potential Employing The In vivo Cytogenetics Method In The Rat (Final Report)	1972	Ajinomoto and Nutrasweet
E31	Sc-19192: An Evaluation Of Mutagenic Potential Employing The Host-Mediated Assay In The Rat (Final Report)	1972	Ajinomoto and Nutrasweet
E32	Sc-18862: 52 Week Oral Toxicity Study In The Infant Monkey	1972	Ajinomoto and Nutrasweet
E33	Sc-18862: Two Year Toxicity Study In The Rat (Annex)	1973	Ajinomoto and Nutrasweet
E34	Sc-18862: Two Year Toxicity Study In The Rat (Final Report)	1973	Ajinomoto and Nutrasweet
E35	Sc-18862: 46 Week Oral Toxicity Study In The Hamster (Supplement N°1 Microscopy For Biological Research, Ltd Histopathology Report - Part I)	1972	Ajinomoto and Nutrasweet
E36	Sc-18862: 46 Week Oral Toxicity Study In The Hamster (Supplement N°1 Microscopy For Biological Research, Ltd Histopathology Report - Part II)	1972	Ajinomoto and Nutrasweet
E37	Sc-19192: Evaluation Of Reproductive Performance In The Rat - Segment I Of The Teratology - Reproduction Profile	1973	Ajinomoto and Nutrasweet
E38	Sc-19192: An Evaluation Of The Embryotoxic And Teratogenic Potential In The Rat - Segment II Of The Teratology- Reproduction Profile	1973	Ajinomoto and Nutrasweet
E39	Sc-18862: A Study Of The Pregnant And Lactating Rat And Of Her Offspring - Segment III Of The Teratology Reproduction Profile	1973	Ajinomoto and Nutrasweet
E40	Sc-18862: An Evaluation Of The Mutagenic Potential In The Rat Employing The Dominant Lethal Assay	1973	Ajinomoto and Nutrasweet
E41	Sc-18862: An Evaluation Of The Mutagenic Potential In The Rat Employing The Dominant Lethal Assay	1973	Ajinomoto and Nutrasweet

E42	Sc-19192: An Evaluation Of The Mutagenic Potential In The Rat Employing The Dominant Lethal Assay	1973	Ajinomoto and Nutrasweet
E43	Sc-18862: An Evaluation Of Mutagenic Potential Employing The In vivo Cytogenetics Method in The Rat (Final Report)	1972	Ajinomoto and Nutrasweet
E44	Sc-18862: An Evaluation Of Mutagenic Potential Employing The Host Mediated Assay In The Rat (Final Report)	1972	Ajinomoto and Nutrasweet
E45	Sc-19192: Acute Toxicity Studies In The Rat, Mouse And Rabbit	1973	Ajinomoto and Nutrasweet
E46	Sc-18862: Acute Toxicity Studies In The Rat, Mouse And Rabbit	1973	Ajinomoto and Nutrasweet
E47	Sc-18862: A Study Of The Pregnant And Lactating Rat And Of Her Offspring - A Segment III Study	1973	Ajinomoto and Nutrasweet
E48	Sc-18862: A Study Of The Pregnant And Lactating Rat And Of Her Offspring - Segment III Of The Teratology Reproduction Profile	1973	Ajinomoto and Nutrasweet
E49	Sc-18862: A Study Of The Pregnant And Lactating Rat And Of Her Offspring (Comparison By Feeding Of Equimolar Quantities Of L-Phenylalanine And/Or L-Aspartic Acid)	1973	Ajinomoto and Nutrasweet
E50	A Study Of The Possible Reaction Of DKP With Aqueous Nitrous Acid	Not reported	Ajinomoto and Nutrasweet
E51	Sc-18862: An Evaluation Of The Embryotoxic And Teratogenic Potential In The Rabbit - A Segment II Study	1973	Ajinomoto and Nutrasweet
E52	Sc-18862: Segment II - An Evaluation Of The Teratogenic Potential In The Rabbit (Final Report)	1973	Ajinomoto and Nutrasweet
E53	Sc-18862: An Evaluation Of The Embryotoxic And Teratogenic Potential In The Rabbit - Segment II Of The Teratology Reproduction Profile	1973	Ajinomoto and Nutrasweet
E54	Sc-18862: An Evaluation Of The Embryotoxic And Teratogenic Potential In The Rabbit - A Segment II Study	1974	Ajinomoto and Nutrasweet
E55	Sc-18862: Segment II - Teratology Study In The Rabbit (Final Report)	1973	Ajinomoto and Nutrasweet
E56	Sc-18862 And 19192 (3:1 Ratio): Segment II Teratology Study In The Rat (Final Report)	1972	Ajinomoto and Nutrasweet
E57	Sc-19192: Segment II Teratology Study In The Rabbit (Final Report)	1972	Ajinomoto and Nutrasweet
E58	Sc-18862: A 26 Week Urinary Bladder Tumorigenicity Study In The Mouse By The Intravesical Pellet Implant Technique (Final Report)	1973	Ajinomoto and Nutrasweet
E59	Sc-19192: A 26 Week Urinary Bladder Tumorigenicity Study In The Mouse By The Intravesical Pellet Implant Technique (Status Report)	1973	Ajinomoto and Nutrasweet
E60	Long Term Tolerance Of Aspartame By Normal Adults	1973	Ajinomoto and Nutrasweet
E61	Long Term Tolerance Of Aspartame By Normal Children And Adolescents	1972	Ajinomoto and Nutrasweet
E62	Sc-18862: An Evaluation Of The Embryotoxic And Teratogenic Potential In The Rabbit - A Segment II Study	1973	Ajinomoto and Nutrasweet
E63	Sc-18862: Segment II An Evaluation Of The Teratogenic Potential In The Rabbit (Final Report)	1973	Ajinomoto and Nutrasweet
E64	Plan For Phase B: Long Term Study Of The Effects On Aspartyl Phenylalanine Methyl Ester (Aspartame) On Obese Adults	1972	Ajinomoto and Nutrasweet

E65	Tolerance Of Aspartame By Diabetic Subjects	1972	Ajinomoto and Nutrasweet
E66	Tolerance Of Loading Doses Of Aspartame By Normal Adolescents	1973	Ajinomoto and Nutrasweet
E67	Long Term Tolerance Of Aspartame By Adult Pku Heterozygotes	1973	Ajinomoto and Nutrasweet
E68	The Metabolism Of Aspartame - Further Studies Of Nitrosation Formation	1973	Ajinomoto and Nutrasweet
E69	The Effect Of Acid Hydrolysis On Sc-18862 - Sc-19192	Not reported	Ajinomoto and Nutrasweet
E70	Sc-18862: Lifetime Toxicity Study In The Rat (Final Report)	1974	Ajinomoto and Nutrasweet
E71	Study Of Possible Nitrosamide Formation From APM And DKP Under Simulated Physiological Conditions	Not reported	Ajinomoto and Nutrasweet
E72	Sc-18862: A 56 Week Urinary Bladder Tumorigenicity Study In The Mouse By The Intravesical Pellet Implant Technique And Addendum To A 26 Week Urinary Bladder Tumorigenicity Study In The Mouse By The Intravesical Pellet Implant Technique (Final Report)	1974	Ajinomoto and Nutrasweet
E73	Sc-19192: A 56 Week Urinary Bladder Tumorigenicity Study In The Mouse By The Intravesical Pellet Implant Technique And Addendum To Sc-19192: A 26 Week Urinary Bladder Tumorigenicity Study In The Mouse By The Intravesical Pellet Implant Technique (Final Report)	1974	Ajinomoto and Nutrasweet
E74	Effects Of Sc-18862 On Lactation In Rats	1973	Ajinomoto and Nutrasweet
E75	Sc-18862: 104 Week Toxicity Study In The Mouse (Final Report)	1974	Ajinomoto and Nutrasweet
E76	Sc-19192: 110 Week Toxicity Study In The Mouse (Final Report)	1974	Ajinomoto and Nutrasweet
E77	Sc-19192: 115 Week Oral Tumorigenicity Study In The Rat Volume I	1974	Ajinomoto and Nutrasweet
E78	Sc-19192: 115 Week Oral Tumorigenicity Study In The Rat Volume II Postmortem Evaluation	1974	Ajinomoto and Nutrasweet
E79	Sc-18862: Segment II An Evaluation Of The Teratogenic Potential In The Rabbit (Final Report)	1974	Ajinomoto and Nutrasweet
E80	The Metabolism Of Aspartame Volume 4 Parts XXIV - XXXI	1974	Ajinomoto and Nutrasweet
E81	Sc-18862: An Evaluation Of Mutagenic Potential Employing The Host- Mediated Assay In The Mouse	1974	Ajinomoto and Nutrasweet
E82	Sc-19192: An Evaluation Of Mutagenic Potential Employing The Host-Mediated Assay In The Mouse	1974	Ajinomoto and Nutrasweet
E83	Sc-18862 Placebo: An Evaluation Of Embryotoxic And Teratogenic Potential Of Specially Prepared Pelleted Diet In The Rabbit	1974	Ajinomoto and Nutrasweet
E84	Sc-18862: Acute Intravenous Toxicity Study In The Rat	1974	Ajinomoto and Nutrasweet
E85	Sc-18862: Acute Intravenous Toxicity Study In The Dog	1974	Ajinomoto and Nutrasweet
E86	Sc-18862: A Supplemental Study Of Dog Brains From A 106 Week Oral Toxicity Study	1973	Ajinomoto and Nutrasweet
E87	Sc-18862 (Aspartame): A Supplemental Study Of Rat Brains From Two Tumorigenicity Studies	1973	Ajinomoto and Nutrasweet

E88	Sc-18862: Experiments In Mated And Pregnant Rhesus Monkeys - A Compilation Of Available Fragmentary Data	1975	Ajinomoto and Nutrasweet
E89	Sc-18862: An Evaluation Of Embryotoxic And Teratogenic Potential In The Mouse	1975	Ajinomoto and Nutrasweet
E90	Sc-18862: An Evaluation Of Embryotoxic And Teratogenic Potential In The Rabbit	1975	Ajinomoto and Nutrasweet
E91	Table of Contents Section I: A. Introduction; B. "Pivotal Animal Safety Studies; C. Overview of Data Reassurance Activities Section II: Review Of Metabolism Studies Section III: Progress Report On Aspartame Studies (University Of Iowa) Section IV: Conclusion	1976	Ajinomoto and Nutrasweet
E92	The Metabolism Of The Methyl Moiety Of Aspartame	1976	Ajinomoto and Nutrasweet
E93	Final Report On Aspartame Studies - Effect Of Aspartame Loading Upon Plasma And Erythrocyte Free Amino Acid Levels In Normal Adult Subjects	1977	Ajinomoto and Nutrasweet
E94	Damage In The Neonatal Mouse Brain Following Ingestion Of Aspartame	Not reported	Ajinomoto and Nutrasweet
E95	Metabolic Studies Of Aspartame And MSG Ingested As A Meal Component (Final Report)	1977	Ajinomoto and Nutrasweet
E96	Plasma Aminograms Of Infants And Adults Fed An Identical High Protein Meal (Final Report)	1977	Ajinomoto and Nutrasweet
E97	Sc-18862: An Evaluation Of Mutagenic Potential Employing The Ames Salmonella/Microsome Assay	1978	Ajinomoto and Nutrasweet
E98	Sc-19192: An Evaluation Of Mutagenic Potential Employing The Ames Salmonella/Microsome Assay (Report Release)	1978	Ajinomoto and Nutrasweet
E99	The Metabolism Of The Aspartyl Moiety Of Aspartame	1978	Ajinomoto and Nutrasweet
E100	The Metabolism Of Aspartate In Infant And Adult Mice	1978	Ajinomoto and Nutrasweet
E101	An Evaluation Of The Mutagenic Potential of Sc-18862 Employing The Ames Salmonella/Microsome Assay (Report Release)	1978	Ajinomoto and Nutrasweet
E102a	Authentication Review Of Selected Materials Submitted To The Food And Drug Administration Relative To Application Of Searle Laboratories To Market Aspartame Volume N°1 Chapter I, II, III	1978	Ajinomoto and Nutrasweet
E102b	Authentication Review Of Selected Materials Submitted To The Food And Drug Administration Relative To Application Of Searle Laboratories To Market Aspartame Volume N°2 Chapter IV, V, VI	1978	Nutrasweet
E102c	Authentication Review Of Selected Materials Submitted To The Food And Drug Administration Relative To Application Of Searle Laboratories To Market Aspartame Volume N°3 From Chapter VII To Chapter XIV	1978	Nutrasweet
E103	Effects Of Aspartame (Sc-18862) On Gonadotropin Secretion In Rats (Biology Document Review And Approval Sign-Off)	1978	Ajinomoto and Nutrasweet
E104	Developmental Assessment Of Infant Macaques Receiving Dietary Aspartame Or Phenylalanine (Final Report)	1979	Ajinomoto and Nutrasweet
E105	Aspartame Administration To The Infant Monkey: Hypothalamic Morphology And Blood Amino Acid Levels	Not reported	Ajinomoto and Nutrasweet

	(Final Report)		
E106	An Evaluation Of The Mutagenic Potential Of Sc-19192 Employing The Ames Salmonella/Microsome Assay (Release Of Report)	1978	Ajinomoto and Nutrasweet
E107	Effect Of Aspartame Loading Upon Plasma And Erythrocyte Free Amino Acid Levels And Blood Methanol Levels In Normal One Year Old Children	1977	Ajinomoto and Nutrasweet
E108	Effect Of Aspartame On Plasma And Red Cell Amino Acids Of Apparently Healthy Female Adults And On Presumed Phenylketonuric Heterozygotes	1978	Ajinomoto and Nutrasweet
E109	Effect Of Aspartame Loading At 100 Mg Per kg Body Weight Upon Plasma And Erythrocyte Levels Of Free Amino Acids In Normal Subjects And Subjects Presumed To Be Heterozygous For Phenylketonuria (Final Report)	1978	Ajinomoto and Nutrasweet
E110	Effect Of Aspartame Loading In Subjects Who Report Symptoms Of Chinese Restaurant Syndrome After Glutamate Ingestion	1979	Ajinomoto and Nutrasweet
E111	Metabolic Studies Of Aspartame And Monosodium Glutamate When Ingested Together As Part Of A Soup Beverage Meal	1979	Ajinomoto and Nutrasweet
E112	Metabolic Studies Of Aspartame And Monosodium Glutamate Ingested As Components Of A Hamburger - Milk Shake Meal System In Normal Adult Subjects	1979	Ajinomoto and Nutrasweet
E169	Pharmacokinetics and metabolism of [¹⁴ C]-SC-19129 and [¹⁴ C]-SC-19200, its free acid, in the dog	1987	Nutrasweet
E170	Pharmacokinetics and metabolism of [¹⁴ C]-SC-19129 and [¹⁴ C]-SC-19200, its free acid, in the rabbit.	1986	Nutrasweet
E171	Pharmacokinetics and metabolism of [¹⁴ C]-SC-19129 in the Rhesus monkeys	1985	Nutrasweet
E172	A Pharmacokinetic Study of [¹⁴ C]-SC-19129 ([¹⁴ C]Beta-aspartame in man.	1987	Nutrasweet
E173	Pharmacokinetics and metabolism of [14C]-Beta-Aspartame in Healthy Subjects	1988	Nutrasweet
UA01	Report Of The Scientific Committee For Food On Sweeteners	1984	Ajinomoto
UA02	Opinion Of An Expert Panel On The Safety Status Of Aspartame As A Non-Nutritive Sweetener (Final)	2006	Ajinomoto
UA03	Food Additive Approval Process By FDA Followed For Aspartame (GAO Report To Senator Metzenbaum)	1987	Ajinomoto
UA04	Histopathological Examination In A Carcinogenicity Study Of Sc-18862 In Slc: Wistar Rats (Final Report)	2006	Ajinomoto

UN01	Final Clinical Research Report. Effects Of Aspartame Ingestion Without Food On Serum Glucose, Insulin, And Glucagon Concentrations In Normal Subjects And Subjects With Diabetes	1988	Nutrasweet
UN02	Aspartame, Brain Amino Acids And Neurochemical Mediators. IISI International Aspartame Workshop, November 17-21, 1986, Marbella, Spain.	1986	Nutrasweet
UN03	Toxicology Studies Done With Beta-Aspartame (Revised). IISI International Aspartame Workshop, November 17-21, 1986, Marbella, Spain.	1986	Nutrasweet
UN04	Clinical Studies To Evaluate Seizures In Children And Adults Allegedly Due To Aspartame Consumption (N15-85-02-109 And N16-85-02-110)	1991	Nutrasweet
UN05	Effect Of Repeated Ingestion Of Beverage Containing Aspartame Or Diketopiperazine On Amino Acid Or Diketopiperazine Concentrations In Plasma And Urine Of Normal Healthy Subjects (N02-84-02-075).	1987	Nutrasweet
UN06	Effect Of Repeated Ingestion Of Beverage Containing Aspartame Or Diketopiperazine On Amino Acid Or Diketopiperazine Concentrations In Subjects Heterozygous For Phenylketonuria (N12-85-02-054).	1987	Nutrasweet
UN07	Multi-Center Clinical Study To Evaluate Allergic Reactions Allegedly Due To Aspartame Consumption (N22-86-02-002)	1992	Nutrasweet
UN08	Safety Of Long-Term Aspartame Administration In Normal Subjects (N07-84-02-093).	1988	Nutrasweet
UN09	A Study Of The Clinical And Biochemical Effects Of Aspartame In Subjects With Chronic Stable Alcoholic Liver Disease (N23-87-02-001)	1991	Nutrasweet
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5659 **ABBREVIATIONS**

5660

ADI	Acceptable Daily Intake
ADME	absorption, distribution, metabolism and excretion
AFC	Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
AFSSA	Agence française de sécurité sanitaire des aliments
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
ANSES	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail
bw	body weight
β-aspartame (β-APM)	1-methyl N-L-β-aspartyl-3-phenyl-L-alaninate
β-AP	β-aspartylphenylalanine
CAS	Chemical Abstracts Service
CE-C ⁴ D	Capillary electrophoresis (CE) with capacitively coupled contactless conductivity detection (C4D)
COC	UK Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment
DKP	5-benzyl-3,6-dioxo-2-piperazine acetic acid
EFSA	European Food Safety Authority
EC	European Commission
EINECS	European Inventory of Existing Commercial chemical Substances
ERF	European Ramazzini Foundation
EU	European Union
FSA	UK Food Standards Agency
GD	Gestational Day
GLP	Good Laboratory Practice
GSH	Glutathione
HPLC	High-performance liquid chromatography
ICGA	International Chewing Gum Association
ISA	International Sweeteners' Association
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD ₅₀	Lethal Dose, 50% i.e. dose that causes death among 50% of

	treated animals
LOAEL	Lowest Observed Adverse Effect Level
MoA	Mode of Action
MPL	Maximum Permitted Level
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
PARNUTS	Food for Particular Nutritional Uses
PKU	Phenylketonuria
QS	<i>Quantum Satis</i>
RSD _R	Relative standard deviation for reproducibility
SCEs	Sister chromatid exchanges
SCF	Scientific Committee on Food
UK COT	UK Committee on toxicity of chemicals in food, consumer products and the environment
US EPA	US Environmental Protection Agency
US FDA	US Food and Drug Administration
WHO	World Health Organization

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- 6540
6541

6542 ANNEXES

6543 **A. SELECTION CRITERIA FOR SCIENTIFIC DATA CONSIDERATION FOR THE RE-EVALUATION OF**
 6544 **ASPARTAME**

6545 The selection criteria for scientific data consideration for the re-evaluation of aspartame described in
 6546 this report will be applied to the existing published and unpublished scientific literature. The literature
 6547 database will include scientific peer reviewed papers and relevant non-peer reviewed papers (such as
 6548 technical reports and published conference proceedings) identified through exhaustive literature
 6549 searches performed using commercial databases and providers (e.g. ISI Web of Knowledge, PubMed),
 6550 available from previous evaluations by EFSA and SCF or obtained as a result of EFSA's recent public
 6551 call for scientific data on aspartame (closure: 30 September 2011).

6552 **Types of studies that will be considered within the criteria for inclusion in the selection process.**

- 6553 a) Experimental studies
- 6554 b) Epidemiological studies in humans
- 6555 c) Case reports supported by medical evidence

6556 **Table 1:** Source and Type of information available that may fall in these categories:
 6557

Peer-reviewed	Not peer-reviewed
Published papers	Unpublished study reports
Meeting abstracts (conference proceedings)	Papers in non-peer reviewed journals or non-peer reviewed e-papers
Published case reports	Meeting abstracts
	Case reports in non-peer reviewed journals

6559 **Tiered Approach for the Selection Process**
 6560

6561 Tier 1. Criteria to be used for the inclusion of scientific papers and reports in the selection process:

- 6562 1. All studies provided by the applicants (including unpublished study reports non peer-
 6563 reviewed) with the original application dossier.
- 6564 2. All studies on the safety and use of aspartame commissioned by national authorities.
- 6565 3. Papers and reports that have been subject to an independent scientific peer-review process (i.e.
 6566 process that scientific journals use to ensure that the articles to be published represent the best
 6567 scholarship available in terms of solid scientific soundness and quality control) and have been
 6568 subsequently published in a scientific journal.
- 6569 4. For non independently peer reviewed papers and reports assessment based on the quality
 6570 control procedures applied and the study designs used with reference to validated standards
 6571 (e.g. OECD protocols and GLP Guidelines).

6572 Tier 2. Criteria to be used for the rejection of papers and reports in the selection process:
 6573

- 6574 1. Insufficient details provided on the performance or outcome of the studies (EFSA, 2009).
- 6575 2. Insufficient information to assess the methodological quality of the studies (EFSA, 2009).

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II. Composition data for methanol from natural food sources

category	FoodEx name	min — max	mean value* (mg/kg)
ANISE BRANDY	Herb liqueur	trace — 563 ppm	281.5
	Spirits	trace — 563 ppm	281.5
ARCTIC BRAMBLE (<i>Rubus arcticus</i> L.)	Blackberries (<i>Rubus fruticosus</i>)	<0.05 ppm in juice	0.05
ARRACK	Punch	25.6 — 750 ppm	387.8
BANANA (<i>Musa sapientum</i> L.)	Bananas	15 ppm	15.0
	Dried bananas	15 ppm	15.0
	Candied fruit, Bananas	15 ppm	15.0
	Nectar, Banana	15 ppm	15.0
	Soft drink, banana flavour	15 ppm	15.0
BEANS	Beans, with pods	1.3 ppm	1.3
	Legumes, beans, green, without pods	1.3 ppm	1.3
	Beans, green, without pods	1.3 ppm	1.3
	Legumes, beans, dried	1.3 ppm	1.3
	Beans	1.3 ppm	1.3
	Broad bean	1.3 ppm	1.3
	Lima bean	1.3 ppm	1.3
	Mung bean	1.3 ppm	1.3
	Urd bean	1.3 ppm	1.3
	Black eye bean	1.3 ppm	1.3
	Horse bean	1.3 ppm	1.3
	Scarlet runner bean	1.3 ppm	1.3
	Rice bean	1.3 ppm	1.3
	Yam bean	1.3 ppm	1.3
	Castor bean	1.3 ppm	1.3
	Beans-based meals	1.3 ppm	1.3
	Beans and meat meal	1.3 ppm	1.3
	Beans and vegetables meal	1.3 ppm	1.3
	Beans, meat, and vegetables meal	1.3 ppm	1.3
	Legume (beans) soup	1.3 ppm	1.3
Prepared legume (beans) salad	1.3 ppm	1.3	
BLACK CURRANTS (<i>Ribes nigrum</i> L.)	Currants (red, black and white) (<i>Ribes nigrum</i> , <i>Ribes rubrum</i>)	4 ppm	4.0
	Jam, Currants (black) (<i>Ribes nigrum</i>)	4 ppm	4.0
	Juice, Blackcurrant	4 ppm	4.0
	Juice concentrate, Currants (black)	4 ppm	4.0
CABBAGE (<i>Brassica oleracea</i>)	Head cabbage (<i>Brassica oleracea</i> convar. <i>capitata</i>)	0.6 ppm	0.6
	Chinese cabbage	0.6 ppm	0.6
CARROT (<i>Daucus carota</i> L.)	Carrots (<i>Daucus carota</i>)	trace — 0.05 ppm	0.03
	Juice, Carrot	trace — 0.05 ppm	0.03
	Apple/carrot juice	trace — 0.05 ppm	0.03
	Multi-fruit-carrot juice	trace — 0.05 ppm	0.03
CIDER (APPLE WINE)	Cider	16 — 248 ppm	132.0
CITRUS FRUITS	Citrus fruits	0 — 213 ppm	106.5

category	FoodEx name	min — max	mean value* (mg/kg)
	Juice, Orange	0 — 213 ppm	106.5
	Juice, Grapefruit	0 — 213 ppm	106.5
	Juice, Lemon	0 — 213 ppm	106.5
	Juice, Lime	0 — 213 ppm	106.5
	Juice, Mixed fruit	0 — 213 ppm	106.5
	Juice concentrate, Oranges	0 — 213 ppm	106.5
	Juice concentrate, Mandarins	0 — 213 ppm	106.5
	Nectar, Orange	0 — 213 ppm	106.5
	Juice, Orange-Grapefruit	0 — 213 ppm	106.5
	Juice, Mandarin-Banana	0 — 213 ppm	106.5
	Juice, Apricot-Orange	0 — 213 ppm	106.5
	Juice, Orange-Peach	0 — 213 ppm	106.5
	Juice, multi-fruit	0 — 213 ppm	106.5
	FIG (<i>Ficus carica</i> L.)	Figs (<i>Ficus carica</i>)	<1 — 5 ppm
Dried figs (<i>Ficus carica</i>)		<1 — 5 ppm	3.0
GIN	Gin	76 ppm	76.0
GRAPE (<i>Vitis</i> species)	Vine leaves (grape leaves) (<i>Vitis euveitidis</i>)	0.032 — 0.04 ppm	<0.04
	Table grapes (<i>Vitis euveitidis</i>)	0.032 — 0.04 ppm	<0.04
	Wine grapes (<i>Vitis euveitidis</i>)	0.032 — 0.04 ppm	<0.04
	Canned fruit, Table grape (<i>Vitis euveitidis</i>)	0.032 — 0.04 ppm	<0.04
	Fruit compote, Table grape (<i>Vitis euveitidis</i>)	0.032 — 0.04 ppm	<0.04
grape juice (Filer and Stegink, 1989)	Juice, Grape	12-680 mg/L	346.0
	Juice concentrate, Grapes	12-680 mg/L	346.0
	Juice, Apple-Grape	12-680 mg/L	346.0
	Juice, Berries-Grapes	12-680 mg/L	346.0
	Fruit soft drink, Grape red	12-680 mg/L	346.0
	Fruit soft drink, Grape white	12-680 mg/L	346.0
	Soft drink, grapefruit flavour	12-680 mg/L	346.0
LENTILS	Lentils, green (<i>Lens culinaris</i> syn. <i>L. esculenta</i>)	4.4 ppm	4.4
	Lentils (<i>Lens culinaris</i> syn. <i>L. esculenta</i>)	4.4 ppm	4.4
MILK and MILK PRODUCTS	Milk and dairy products	<0.009 ppm	<0.005
	Butter	<0.009 ppm	<0.005
	Butter oil	<0.009 ppm	<0.005
	Ghee	<0.009 ppm	<0.005
PASSIFLORA SPECIES	Passion fruit (<i>Passiflora edulis</i>)	4 ppm	4.0
	Juice, Passion fruit	4 ppm	4.0
PEAR BRANDY	Fruit liqueur	4300 — 9300 ppm	6800.0
	Brandy	4300 — 9300 ppm	6800.0
	Spirits made from fruits	4300 — 9300 ppm	6800.0
PEAS (<i>Pisum sativum</i> L.)	Peas, with pods (<i>Pisum sativum</i>)	1 ppm	1.0
	Mushy peas (<i>Pisum sativum</i>)	1 ppm	1.0
	Peas, green, without pods (<i>Pisum sativum</i>)	1 ppm	1.0

category	FoodEx name	min — max	mean value* (mg/kg)
	Peas (<i>Pisum sativum</i>)	1 ppm	1.0
RASPBERRY, BLACKBERRY and BOYSENBERRY	Boysenberry (<i>Rubus ursinus x idaeus</i>)	7 — 16 ppm	11.5
	Juice, Blackberry	7 — 16 ppm	11.5
	Fruit soft drink, Raspberry	7 — 16 ppm	11.5
	Soft drink, raspberry flavour	7 — 16 ppm	11.5
RUM	Rum	0.08 — 36 ppm	18.0
SHERRY	Sherry	31 — 163 ppm	97.0
SOYBEAN (Glycine max. L. merr.)	Soya beans	0.02 — 6 ppm	3.0
	Soya beans flour	0.02 — 6 ppm	3.0
STRAWBERRY (Fragaria species)	Strawberries (<i>Fragaria × ananassa</i>)	0.01 — 5 ppm	2.5
	Jam, Strawberries	0.01 — 5 ppm	2.5
	Juice concentrate, Strawberries	0.01 — 5 ppm	2.5
	Juice, Strawberry-Cherry	0.01 — 5 ppm	2.5
TEA	Tea (dried leaves and stalks, fermented or otherwise of <i>Camellia sinensis</i>)	0.01 — 1.8 ppm	0.9
	Tea (dried leaves and stalks, fermented or otherwise of <i>Camellia sinensis</i>), decaffeinated	0.01 — 1.8 ppm	0.9
	Tea (Infusion)	0.01 — 1.8 ppm	0.9
TEQUILA (Agave tequilana)	Spirits made from vegetables (Tequila)	500 — 1000 ppm	750.0
TOMATO (Lycopersicon esculentum Mill.)	Tomatoes (<i>Lycopersicon esculentum</i>)	2.7 — 560 ppm	281.4
	Tomato purée	2.7 — 560 ppm	281.4
	Sun-dried tomatoes	2.7 — 560 ppm	281.4
	Juice, Tomato	2.7 — 560 ppm	281.4
	Juice, Tomato-Vegetable	2.7 — 560 ppm	281.4
	Juice, Tomato-Carrot	2.7 — 560 ppm	281.4
	Tomato ketchup	2.7 — 560 ppm	281.4
	Tomato chutney	2.7 — 560 ppm	281.4
WHISKY	Whisky	15 — 160 ppm	87.5
WINE	Wine	0.086 — 230 ppm	115.0

6588 *mean values have been calculated by the Panel for the purpose of estimating background chronic intake of methanol from
6589 natural food occurrence which is based on assumption that distribution of composition data per category (only when range is
6590 expressed) is following a uniform distribution.

6591 Analytical values from VCF., 28th March 2012 and Filer and Stegink, 1989.

6592

III. Composition data for methanol from pectine degradation

Matrix description	pectin content (g/100g)	methanol (mg/kg)
Vegetables and vegetable products (including fungi)	0.66	594
Root vegetables	0.86	774
Bulb vegetables	0.66	594
Fruiting vegetables	0.67	603
Brassica vegetables	0.66	594
Leaf vegetables	0.66	594
Legume vegetables	0.45	405
Stem vegetables (Fresh)	0.66	594
Vegetable products	0.66	594
Fungi, cultivated	0.45	405
Fungi, wild, edible	0.45	405
Starchy roots and tubers	0.695	625.5
Legumes, nuts and oilseeds	0.45	405
Fruit and fruit products	0.59	531
Citrus fruits	0.59	531
Grapefruit (<i>Citrus paradisi</i>)	0.445	400.5
Oranges (<i>Citrus sinensis</i>)	0.59	531
Lemons (<i>Citrus limon</i>)	0.63	567
Limes (<i>Citrus aurantifolia</i>)	0.63	567
Mandarins (<i>Citrus reticulata</i>)	0.59	531
Pomelo (<i>Citrus grandis</i>)	0.445	400.5
Pome fruits	0.565	508.5
Apple (<i>Malus domestica</i>)	0.565	508.5
Pear (<i>Pyrus communis</i>)	0.565	508.5
Quince (<i>Cydonia oblonga</i>)	0.565	508.5
Medlar (<i>Mespilus germanica</i>)	0.565	508.5
Loquat (<i>Eriobotrya japonica</i>)	0.565	508.5
Hawthorn (<i>Crataegus</i> and <i>Rhaphiolepis</i>)	0.565	508.5
Rowan (<i>Sorbus</i>)	0.565	508.5
Service tree (<i>Sorbus domestica</i>)	0.565	508.5
Nashi pear (<i>Pyrus pyrifolia</i>)	0.565	508.5
Stone fruits	0.635	571.5
Apricots (<i>Prunus armeniaca</i>)	0.87	783
Plums (<i>Prunus domestica</i>)	0.635	571.5
Damson plum (<i>Prunus domestica</i> var <i>institia</i>)	0.635	571.5
Mirabelle (<i>Prunus domestica</i> var <i>syriaca</i>)	0.635	571.5
Greengage (<i>Prunus domestica</i> var <i>italica</i>)	0.635	571.5
Sweet cherry (<i>Prunus avium</i>)	0.4	360
Sour cherry (<i>Prunus cerasus</i>)	0.4	360
Chickasaw plum (<i>Prunus angustifolia</i>)	0.635	571.5
Susina (<i>Prunus salicina</i>)	0.635	571.5
Sloe (<i>Prunus spinosa</i>)	0.635	571.5
Peaches (<i>Prunus persica</i>)	0.635	571.5
Chokecherry (<i>Prunus virginiana</i>)	0.4	360
Berries and small fruits	0.55	495
Table grapes (<i>Vitis euvtis</i>)	0.75	675
Wine grapes (<i>Vitis euvtis</i>)	0.75	675
Strawberries (<i>Fragaria</i> × <i>ananassa</i>)	0.47	423
Blackberries (<i>Rubus fruticosus</i>)	0.55	495
Dewberries (<i>Rubus ceasius</i>)	0.7	630
Raspberries (<i>Rubus idaeus</i>)	0.49	441
Boysenberry (<i>Rubus ursinus</i> × <i>idaeus</i>)	0.55	495
Blueberries (<i>Vaccinium corymbosum</i>)	0.55	495
Cranberries (<i>Vaccinium macrocarpon</i>)	0.55	495
Currants (red, black and white) (<i>Ribes nigrum</i> , <i>Ribes</i>)	0.55	495
Gooseberries (<i>Ribes uva-crispa</i>)	0.55	495
Rose hips (<i>Rosa canina</i>)	0.55	495

Matrix description	pectin content (g/100g)	methanol (mg/kg)
Mulberries (<i>Morus spp.</i>)	0.55	495
Azarole (mediteranean medlar) (<i>Crataegus azarolus</i>)	0.55	495
Elderberries (<i>Sambucus nigra</i>)	0.55	495
Cloudberry (<i>Rubus chamaemorus</i>)	0.55	495
Loganberry (<i>Rubus loganobaccus</i>)	0.55	495
Salmonberry (<i>Rubus spectabilis</i>)	0.55	495
Thimbleberry (<i>Rubus parviflorus</i>)	0.55	495
Wineberry (<i>Rubus phoenicolasius</i>)	0.55	495
Bearberry (<i>Arctostaphylos spp.</i>)	0.55	495
Bilberry or whortleberry (<i>Vaccinium spp.</i>)	0.55	495
Crowberry (<i>Empetrum spp.</i>)	0.55	495
Cranberry (<i>Vaccinium spp.</i>)	0.55	495
Huckleberry (<i>Vaccinium spp.</i>)	0.55	495
Lingonberry (<i>Vaccinium vitis-idaea</i>)	0.55	495
Strawberry Tree (<i>Arbutus unedo</i>), not to be confused with	0.55	495
Jaboticaba (Brazilian grape tree) (<i>Myrciaria cauliflora</i>)	0.55	495
Physalis (<i>Physalis peruviana</i>)	0.55	495
Sea buckthorn (<i>Hippophae rhamnoides</i>)	0.55	495
Miscellaneous fruits	0.54	486
Dates (<i>Phoenix dactylifera</i>)	0.54	486
Figs (<i>Ficus carica</i>)	0.54	486
Table olives (<i>Olea europaea</i>)	0.54	486
Kumquats (<i>Fortunella species</i>)	0.54	486
Carambola (<i>Averrhoa carambola</i>)	0.27	243
Persimmon (Sharon fruit) (<i>Diospyros kaki</i>)	0.54	486
Jambolan (java plum) (<i>Syzygium cumini</i>)	0.54	486
Kiwi (<i>Actinidia deliciosa</i> syn. <i>A. chinensis</i>)	0.54	486
Lychee (Litchi) (<i>Litchi chinensis</i>)	0.48	432
Passion fruit (<i>Passiflora edulis</i>)	0.54	486
Prickly pear (cactus fruit) (<i>Opuntia ficus-indica</i>)	0.54	486
Star apple (<i>Chrysophyllum cainito</i>)	0.54	486
American persimmon (<i>Virginia kaki</i>) (<i>Diospyros virginiana</i>)	0.54	486
Avocados (<i>Persea americana</i>)	0.54	486
Bananas (<i>Musa × paradisiaca</i>)	0.73	657
Mangoes (<i>Mangifera indica</i>)	0.48	432
Papaya (<i>Carica papaya</i>)	0.6	540
Pomegranate (<i>Punica granatum</i>)	0.54	486
Cherimoya (<i>Annona cherimola</i>)	0.54	486
Guava (<i>Psidium guajava</i>)	1.04	936
Pineapples (<i>Ananas comosus</i>)	0.54	486
Bread fruit (jackfruit) (<i>Artocarpus altilis</i>)	0.54	486
Durian (<i>Durio zibethinus</i>)	0.54	486
Soursop (guanabana) (<i>Annona muricata</i>)	0.54	486
Anona (cherimoya) (<i>Annona cherimola</i>)	0.54	486
Babaco (<i>Carica pentagona</i> Heilb., sin. <i>Vasconcellea x</i>)	0.54	486
Coco plum (<i>Chrysobalanus icaco</i>)	0.54	486
Feijoa, Pineapple Guava (<i>Feijoa sellowiana</i>)	0.54	486
Pawpaw (<i>Asimina</i>)	0.54	486
Chayote (<i>Sechium edule</i>)	0.54	486
Rambutan (<i>Nephelium lappaceum</i>)	0.54	486
Carissa (<i>Carissa</i> sp.)	0.54	486
Jujube (<i>Ziziphus zizyphus</i>)	0.54	486
Longan fruit (<i>Dimocarpus longan</i>)	0.2	180
Malabar plum (jambu) (<i>Syzygium jambos</i>)	0.54	486

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IV. Degradation of DKP from aspartame (from data reported in the technical section and from the MPLs/Use levels of aspartame as reported from table 6)

Cat N°	Foods	restrictions/exception	MPLs (mg/l or mg/kg)	Used levels (mg/l or mg/kg)	% DKP	mg DKP from MPLs	mg DKP from used levels
1.4	Flavoured fermented milk products including heat treated products	only energy-reduced products or with no added sugar	1000	1000	5	45	45
3	Edible ices	only energy-reduced or with no added sugar	800	800	5	36	36
4.2.2	Fruit and vegetables in vinegar, oil, or brine	only sweet-sour preserves of fruit and vegetables	300	300	24	64	64
4.2.3	Canned or bottled fruit and vegetables	only fruit energy-reduced or with no added sugar	1000	1000	24	214	214
4.2.4.1	Fruit and vegetable preparations excluding compote	only energy-reduced	1000	1000	24	214	214
4.2.5.1	Extra jam and extra jelly as defined by Directive 2001/113/EEC	only energy-reduced jams jellies and marmalades	1000	1000	24	214	214
4.2.5.2	Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EEC	only energy-reduced jams, jellies and marmalades	1000	1000	24	214	214
4.2.5.3	Other similar fruit or vegetable spreads	only dried-fruit-based sandwich spreads, energy-reduced or with no added sugar	1000	1000	24	214	214
5.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	only energy-reduced or with no added sugars	2000	2000	10	178	178
5.2	Other confectionery including breath refreshing microsweets	only cocoa or dried fruit based, energy reduced or with no added sugar	2000	1000	10	178	89
5.2	Other confectionery including breath refreshing microsweets	only cocoa, milk, dried fruit or fat based sandwich spreads, energy-reduced or with no added sugar	1000		10	89	89
5.2	Other confectionery including breath refreshing microsweets	only starch based confectionery energy reduced or with no added sugar	2000		10	178	89
5.2	Other confectionery including breath refreshing microsweets	only confectionery with no added sugar	1000		10	89	89
5.2	Other confectionery including breath refreshing microsweets	only breath-freshening micro-sweets, with no added sugar	6000		10	535	89
5.2	Other confectionery including breath refreshing microsweets	only strongly flavoured freshening throat pastilles with no added sugar	2000		10	178	89
5.3	Chewing gum	only with added sugars or polyols, as flavour enhancer	2500	1450	0.1	2	1
5.3	Chewing gum	only with no added sugar	5500	5420	0.1	5	5
5.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	only starch based confectionery energy reduced or with no added sugar	2000	2000	10	178	178
5.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	only confectionery with no added sugar	1000	1000	10	89	89
5.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	only cocoa or dried fruit based, energy reduced or with no added sugar	2000	2000	10	178	178
5.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	only sauces	350	350	10	31	31

Cat N°	Foods	restrictions/exception	MPLs (mg/l or mg/kg)	Used levels (mg/l or mg/kg)	% DKP	mg DKP from MPLs	mg DKP from used levels
6.3	Breakfast cereals	only breakfast cereals with a fibre content of more than 15 %, and containing at least 20 % bran, energy reduced or with no added sugar	1000	1000	0.2	2	2
7.2	Fine bakery wares	only essoblaten - wafer paper	1000	1000	0.2	2	2
7.2	Fine bakery wares	only fine bakery products for special nutritional uses	1700	1700	0.2	3	3
9.2.	Processed fish and fishery products including mollusks and crustaceans	only sweet-sour semi-preserves of fish and marinades of fish, crustaceans and molluscs	300	300	5	13	13
11.4.1	Table-top Sweeteners in liquid form		QS	500000	24	106932	106932
11.4.2	Table-top Sweeteners in powder form		QS	500000	0.2	891	891
11.4.3	Table-top Sweeteners in tablets		QS	500000	0.2	891	891
12.4	Mustard		350	350	24	75	75
12.5	Soups and broths	only energy-reduced soups	110	110	24	24	24
12.6	Sauces		350	350	24	75	75
12.7	Salads and savoury based sandwich spreads	only <i>Feinkostsalat</i>	350	350	24	75	75
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	Products in this category can also use additives that are allowed in the corresponding food counterparts categories	1000	1000	24	214	214
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)		800	800	24	171	171
14.1.3	Fruit nectars as defined by Council Directive 2001/112/EC and vegetable nectars and similar products	only energy-reduced or with no added sugar	600	75	24	128	16
14.1.4	Flavoured drinks	only energy reduced or with no added sugar	600	600	24	128	128
14.2.1	Beer and malt beverages	only alcohol-free beer or with an alcohol content not exceeding 1,2 % vol; 'Bière de table/Tafelbier/Table beer' (original wort content less than 6 %) except for 'Oberjähriges Einfachbier'; Beers with a minimum acidity of 30 milli-equivalents expressed as NaOH; Brown beers of the 'oud bruin' type	600	600	0.7	4	4
14.2.1	Beer and malt beverages	only energy-reduced beer	25	25	0.7	0.2	0.2
14.2.3	Cider and perry		600	600	0.7	4	4
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15 % of alcohol		600	600	0.7	4	4
15.1	Potato-, cereal-, flour- or starch-based snacks		500	500	24	107	107
15.2	Processed nuts		500	500	24	107	107
16	Desserts excluding products covered in category 1, 3 and 4	only energy-reduced or with no added sugar	1000	1000	24	214	214
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms excluding chewable forms		2000	2245	0.2	4	4

Cat N°	Foods	restrictions/exception	MPLs (mg/l or mg/kg)	Used levels (mg/l or mg/kg)	% DKP	mg DKP from MPLs	mg DKP from used levels
17.2	Food supplements supplied in a liquid form		600		24	128	480
17.3	Food supplements supplied in a syrup-type or chewable form		5500		24	1176	480

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**C. SUMMARY OF TOTAL ESTIMATED EXPOSURE TO ASPARTAME PER AGE CLASS AND SURVEY*:
MEAN AND HIGH LEVEL (MG/KG BW/DAY)**

	MPLs		Use levels	
	Mean	High level	Mean	High level
Toddlers				
Belgium (Regional_Flanders)	16.3		16.3	
Bulgaria (Nutrichild)	3.2	11.8	1.6	7.5
Finland (DIPP)	6.3	21.2	6.3	21.2
Germany (Donald 2006_2008)	4.3	13.2	4.3	13.2
Italy (INRAN_SCAI_2005_06)	3.9		3.5	
The Netherlands (VCP_Kids)	14.2	36.9	14.1	36.0
Spain (enKid)	8.3		8.3	
Children				
Belgium (Regional_Flanders)	11.4	27.1	11.4	27.1
Bulgaria (Nutrichild)	2.9	9.3	1.8	6.3
Czech Republic (SISP04)	5.2	12.1	5.2	12.1
Denmark (Danish Dietary Survey)	3.7	8.7	3.7	8.7
Finland (DIPP)	6.8	17.2	6.8	16.9
Finland (STRIP)	5.3	11.7	5.2	11.7
France (INCA 2)	6.5	15.9	6.4	15.3
Germany (Donald 2006_2008)	6.4	14.9	6.3	14.2
Greece (Regional_Crete)	2.7	8.0	2.7	8.0
Italy (INRAN_SCAI_2005_06)	2.3	7.1	2.2	7.1
Latvia (EFSA_TEST)	3.4	8.3	3.4	8.3
The Netherlands (VCP_Kids)	12.8	32.9	12.6	32.4
Spain (enKid)	6.0	17.3	6.0	17.3
Spain (Nut_Ink05)	5.4	14.2	5.4	14.2
Sweden (NFA)	7.0	16.0	6.9	16.0
Adolescents				
Belgium (Diet_National_2004)	2.7	7.2	2.7	7.2
Cyprus (Childhealth)	0.8	2.3	0.8	2.3
Czech Republic (SISP04)	2.7	6.9	2.7	6.9
Denmark (Danish Dietary Survey)	2.0	5.3	2.0	5.3
France (INCA 2)	3.1	7.6	3.1	7.2
Germany (National_Nutrition_Survey_II)	4.0	13.3	4.0	13.2
Italy (INRAN_SCAI_2005_06)	1.1	4.1	1.1	4.1
Latvia (EFSA_TEST)	2.1	5.6	2.1	5.6
Spain (AESAN_FIAB)	1.2	3.1	1.2	3.1
Spain (enKid)	2.4	6.3	2.4	6.3
Spain (Nut_Ink05)	3.1	8.0	3.1	8.0
Sweden (NFA)	3.5	9.5	3.5	9.5

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	MPLs		Use levels	
	Mean	High level	Mean	High level
Adults				
Belgium (Diet_National_2004)	3.8	14.0	3.8	14.0
Czech Republic (SISP04)	1.1	3.4	1.1	3.4
Denmark (Danish_Dietary_Survey)	1.1	3.3	1.1	3.2
Finland (FINDIET_2007)	2.1	6.5	2.1	6.3
France (INCA2)	3.4	8.9	3.4	8.9
Germany (National_Nutrition_Survey_II)	4.6	17.3	4.6	17.1
Hungary (National_Repr_Surv)	0.9	3.1	0.7	2.4
Ireland (NSIFCS)	3.1	8.2	3.1	8.2
Italy (INRAN_SCAI_2005_06)	0.9	3.1	0.9	3.1
Latvia (EFSA_TEST)	1.2	3.2	1.2	3.2
The Netherlands (DNFCS_2003)	8.6	27.5	8.5	27.5
Spain (AESAN)	1.5	5.0	1.4	5.0
Spain (AESAN_FIAB)	0.8	2.5	0.7	2.5
Sweden (Riksmaten_1997_98)	2.3	5.9	1.6	4.1
United Kingdom (NDNS)	4.3	20.6	4.2	20.0
The elderly				
Belgium (Diet_National_2004)	4.4	23.5	4.4	23.5
Denmark (Danish_Dietary_Survey)	0.8	2.6	0.8	2.6
Finland (FINDIET_2007)	1.4	4.7	1.3	4.7
France (INCA2)	2.8	11.3	2.8	11.3
Germany (National_Nutrition_Survey_II)	4.1	18.7	4.1	18.7
Hungary (National_Repr_Surv)	0.5	1.5	0.4	1.4
Italy (INRAN_SCAI_2005_06)	0.8	2.7	0.8	2.7

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6604 **D. SUMMARY OF TOTAL ESTIMATED EXPOSURE TO METHANOL FROM ALL SOURCES PER AGE CLASS AND SURVEY*: MEAN AND HIGH LEVEL (MG/KG**
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	from endogenous pathways (basal + pectine-derived)		from natural food occurrence		from food additive (using aspartame MPLs)		Total exposure from all sources	
	Mean	High level	Mean	High level	Mean	High level	Mean	High level
Toddlers								
Belgium (Regional_Flanders)	15.5		1.4		1.6		18.6	
Bulgaria (Nutrichild)	17.0	31.8	1.4	3.8	0.3	1.2	18.7	34.6
Finland (DIPP)	18.0	33.1	0.2	0.9	0.6	2.1	18.9	35.1
Germany (Donald 2006_2008)	14.7	29.1	0.5	1.9	0.4	1.3	15.6	29.9
Italy (INRAN_SCAI_2005_06)	15.4		1.0		0.4		16.8	
The Netherlands (VCP_Kids)	13.4	24.2	0.6	2.6	1.4	3.7	15.5	26.3
Spain (enKid)	13.7		0.2		0.8		14.7	
Children								
Belgium (Regional_Flanders)	13.5	24.4	1.1	3.1	1.1	2.7	15.8	28.1
Bulgaria (Nutrichild)	16.0	29.0	1.4	3.3	0.3	0.9	17.6	31.8
Czech Republic (SISP04)	13.1	23.2	0.3	0.9	0.5	1.2	13.9	24.5
Denmark (Danish Dietary Survey)	13.1	24.6	0.6	1.4	0.4	0.9	14.0	25.7
Finland (DIPP)	13.0	23.6	0.4	1.4	0.7	1.7	14.1	25.4
Finland (STRIP)	9.4	16.7	0.4	1.1	0.5	1.2	10.4	18.1
France (INCA 2)	11.2	20.1	0.6	1.5	0.6	1.6	12.4	21.8
Germany (Donald 2006_2008)	11.5	21.1	0.5	1.8	0.6	1.5	12.7	22.8
Greece (Regional_Crete)	9.8	18.0	0.8	2.1	0.3	0.8	10.8	19.6
Italy (INRAN_SCAI_2005_06)	13.2	23.5	1.0	2.1	0.2	0.7	14.4	25.4
Latvia (EFSA_TEST)	10.1	20.2	0.3	1.2	0.3	0.8	10.7	21.2
The Netherlands (VCP_Kids)	11.4	21.1	0.6	2.1	1.3	3.3	13.2	23.5
Spain (enKid)	11.5	24.1	0.3	1.1	0.6	1.7	12.5	25.5
Spain (Nut_Ink05)	10.3	19.4	0.5	1.6	0.5	1.4	11.4	21.2
Sweden (NFA)	11.0	20.3	0.3	1.1	0.7	1.6	12.1	21.9

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	from endogenous pathways (basal + pectine)		from natural sources		from food additive (using aspartame MPLs)		total exposure to all sources of methanol	
	Mean	High level	Mean	High level	Mean	High level	Mean	High level
Adolescents								
Belgium (Diet_National_2004)	8.1	14.8	0.5	1.2	0.3	0.7	8.8	15.9
Cyprus (Childhealth)	8.6	15.1	0.5	1.4	0.1	0.2	9.2	16.5
Czech Republic (SISP04)	10.5	19.5	0.2	0.7	0.3	0.7	11.0	20.2
Denmark (Danish Dietary Survey)	9.4	16.9	0.4	1.0	0.2	0.5	10.1	17.8
France (INCA 2)	8.5	15.4	0.4	0.9	0.3	0.8	9.2	16.4
Germany (National_Nutrition_Survey_II)	7.6	14.3	0.4	1.3	0.4	1.3	8.4	15.5
Italy (INRAN_SCAI_2005_06)	10.1	17.5	0.6	1.3	0.1	0.4	10.8	18.8
Latvia (EFSA_TEST)	8.8	17.3	0.3	0.8	0.2	0.6	9.3	18.1
Spain (AESAN_FIAB)	9.1	15.6	0.3	0.8	0.1	0.3	9.5	16.4
Spain (enKid)	9.1	16.1	0.2	0.7	0.2	0.6	9.5	17.2
Spain (Nut_Ink05)	8.4	15.3	0.5	1.4	0.3	0.8	9.2	16.7
Sweden (NFA)	8.4	15.1	0.2	0.6	0.3	1.0	8.9	15.8
Adults								
Belgium (Diet_National_2004)	8.2	14.8	0.5	1.4	0.4	1.4	9.1	16.3
Czech Republic (SISP04)	8.2	14.3	0.3	0.8	0.1	0.3	8.5	15.1
Denmark (Danish_Dietary_Survey)	9.0	16.2	0.4	1.0	0.1	0.3	9.6	16.9
Finland (FINDIET_2007)	8.5	15.4	0.6	1.6	0.2	0.7	9.3	16.8
France (INCA2)	8.6	15.3	0.4	1.1	0.3	0.9	9.3	16.5
Germany (National_Nutrition_Survey_II)	8.1	14.9	0.4	1.2	0.5	1.7	8.9	16.4
Hungary (National_Repr_Surv)	9.1	15.8	0.3	0.8	0.1	0.3	9.5	16.3
Ireland (NSIFCS)	9.6	16.7	0.3	0.9	0.3	0.8	10.2	17.9
Italy (INRAN_SCAI_2005_06)	9.6	16.3	0.6	1.1	0.1	0.3	10.2	17.4
Latvia (EFSA_TEST)	7.9	14.9	0.3	0.9	0.1	0.3	8.4	15.7
The Netherlands (DNFCS_2003)	7.9	14.1	0.4	0.9	0.9	2.7	9.1	16.2
Spain (AESAN)	9.1	16.6	0.4	1.2	0.1	0.5	9.7	17.9
Spain (AESAN_FIAB)	9.7	16.9	0.6	1.3	0.1	0.3	10.3	18.2
Sweden (Riksmaten_1997_98)	8.1	14.2	0.3	0.7	0.2	0.6	8.6	15.1
United Kingdom (NDNS)	8.3	14.4	0.4	1.0	0.4	2.1	9.1	16.1

	from endogenous pathways (basal + pectine)		from natural sources		from food additive (using aspartame MPLs)		total exposure to all sources of methanol	
	Mean	High level	Mean	High level	Mean	High level	Mean	High level
The elderly								
Belgium (Diet_National_2004)	8.6	15.3	0.4	1.3	0.4	2.4	9.4	17.2
Denmark (Danish_Dietary_Survey)	9.5	16.7	0.5	1.1	0.1	0.3	10.0	17.5
Finland (FINDIET_2007)	8.5	15.0	0.4	1.4	0.1	0.5	9.0	16.5
France (INCA2)	9.5	16.4	0.4	1.0	0.3	1.1	10.2	17.2
Germany (National_Nutrition_Survey_II)	8.5	15.3	0.3	1.0	0.4	1.9	9.3	16.6
Hungary (National_Repr_Surv)	9.3	15.3	0.2	0.6	0.0	0.2	9.5	15.8
Italy (INRAN_SCAI_2005_06)	10.0	17.1	0.6	1.2	0.1	0.3	10.7	17.9

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*The different methodologies of European dietary surveys included in the EFSA Comprehensive Database are fully described in the Guidance on the use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment (EFSA, 2011a). A summary is available p.11, Table 1 of the guidance.

6612 E. SUMMARY OF TOTAL ESTIMATED EXPOSURE TO ASPARTIC ACID, PHENYLALANINE AND
6613 DKP (FROM THE USE OF ASPARTAME MPLS) PER AGE CLASS AND SURVEY*: MEAN AND
6614 HIGH LEVEL (MG/KG BW/DAY)

	Aspartic acid		PHE		DKP	
	Mean	High level	Mean	High level	Mean	High level
Toddlers						
Belgium (Regional_Flanders)	7.4		9.2		1.9	
Bulgaria (Nutrichild)	1.5	5.3	1.8	6.6	0.5	2.0
Finland (DIPP)	2.8	9.6	3.5	11.9	0.5	1.5
Germany (Donald 2006_2008)	2.0	5.9	2.4	7.4	0.4	1.2
Italy (INRAN_SCAI_2005_06)	1.8		2.2		0.3	
The Netherlands (VCP_Kids)	6.4	16.7	8.0	20.7	1.5	4.1
Spain (enKid)	3.7		4.6		0.7	
Children						
Belgium (Regional_Flanders)	5.2	12.2	6.4	15.2	1.1	3.2
Bulgaria (Nutrichild)	1.3	4.2	1.6	5.2	0.4	1.7
Czech Republic (SISP04)	2.3	5.5	2.9	6.8	0.4	1.1
Denmark (Danish Dietary Survey)	1.7	3.9	2.1	4.9	0.4	1.0
Finland (DIPP)	3.1	7.8	3.8	9.7	0.7	1.6
Finland (STRIP)	2.4	5.3	3.0	6.6	0.7	1.6
France (INCA 2)	2.9	7.2	3.6	8.9	1.0	2.7
Germany (Donald 2006_2008)	2.9	6.7	3.6	8.4	0.6	1.9
Greece (Regional_Crete)	1.2	3.6	1.5	4.5	0.3	0.8
Italy (INRAN_SCAI_2005_06)	1.0	3.2	1.3	4.0	0.2	0.5
Latvia (EFSA_TEST)	1.5	3.8	1.9	4.7	0.5	1.6
The Netherlands (VCP_Kids)	5.8	14.9	7.2	18.4	1.3	3.2
Spain (enKid)	2.7	7.8	3.4	9.7	0.5	1.6
Spain (Nut_Ink05)	2.4	6.4	3.0	8.0	0.6	1.6
Sweden (NFA)	3.1	7.3	3.9	9.0	0.7	1.5
Adolescents						
Belgium (Diet_National_2004)	2.7	7.2	2.7	7.2	0.4	1.2
Cyprus (Childhealth)	0.8	2.3	0.8	2.3	0.1	0.3
Czech Republic (SISP04)	2.7	6.9	2.7	6.9	0.2	0.6
Denmark (Danish Dietary Survey)	2.0	5.3	2.0	5.3	0.2	0.7
France (INCA 2)	3.1	7.6	3.1	7.2	0.5	1.3
Germany (National_Nutrition_Survey_II)	4.0	13.3	4.0	13.2	0.7	2.5
Italy (INRAN_SCAI_2005_06)	1.1	4.1	1.1	4.1	0.1	0.3
Latvia (EFSA_TEST)	2.1	5.6	2.1	5.6	0.3	0.9
Spain (AESAN_FIAB)	1.2	3.1	1.2	3.1	0.1	0.4
Spain (enKid)	2.4	6.3	2.4	6.3	0.2	0.6
Spain (Nut_Ink05)	3.1	8.0	3.1	8.0	0.5	1.5
Sweden (NFA)	3.5	9.5	3.5	9.5	0.3	0.9

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	Aspartic acid		PHE		DKP	
	Mean	High level	Mean	High level	Mean	High level
Adults						
Belgium (Diet_National_2004)	3.8	14.0	3.8	14.0	0.7	2.8
Czech Republic (SISP04)	1.1	3.4	1.1	3.4	0.1	0.3
Denmark (Danish_Dietary_Survey)	1.1	3.3	1.1	3.2	0.1	0.4
Finland (FINDIET_2007)	2.1	6.5	2.1	6.3	0.3	0.9
France (INCA2)	3.4	8.9	3.4	8.9	0.6	1.7
Germany (National_Nutrition_Survey_II)	4.6	17.3	4.6	17.1	0.8	3.4
Hungary (National_Repr_Surv)	0.9	3.1	0.7	2.4	0.1	0.5
Ireland (NSIFCS)	3.1	8.2	3.1	8.2	0.5	1.1
Italy (INRAN_SCAI_2005_06)	0.9	3.1	0.9	3.1	0.1	0.3
Latvia (EFSA_TEST)	1.2	3.2	1.2	3.2	0.2	0.5
The Netherlands (DNFCS_2003)	8.6	27.5	8.5	27.5	1.6	5.5
Spain (AESAN)	1.5	5.0	1.4	5.0	0.2	0.7
Spain (AESAN_FIAB)	0.8	2.5	0.7	2.5	0.1	0.3
Sweden (Riksmaten_1997_98)	2.3	5.9	1.6	4.1	0.4	1.0
United Kingdom (NDNS)	4.3	20.6	4.2	20.0	0.8	4.1
The elderly						
Belgium (Diet_National_2004)	4.4	23.5	4.4	23.5	0.8	4.8
Denmark (Danish_Dietary_Survey)	0.8	2.6	0.8	2.6	0.1	0.2
Finland (FINDIET_2007)	1.4	4.7	1.3	4.7	0.2	0.8
France (INCA2)	2.8	11.3	2.8	11.3	0.5	2.0
Germany (National_Nutrition_Survey_II)	4.1	18.7	4.1	18.7	0.8	3.8
Hungary (National_Repr_Surv)	0.5	1.5	0.4	1.4	0.1	0.2
Italy (INRAN_SCAI_2005_06)	0.8	2.7	0.8	2.7	0.1	0.3

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*The different methodologies of European dietary surveys included in the EFSA Comprehensive Database are fully described in the Guidance on the use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment (EFSA, 2011a). A summary is available p.11, Table 1 of the guidance.

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6620 **F. TABLES ON ACUTE TOXICITY STUDIES ON ASPARTAME AND DKP**

6621 1. Aspartame

	Animals	Route	Dose range	Reported Result	Comments and reference(s)
Oral					
Rat	Male Sprague Dawley, Charles River (n=6/group)	p.o. as 15-25% suspension in 1% Tween 80	Up to 5000 mg/kg	No deaths within 7 days at any dose	Motor and behavioural activities unremarkable. Ref: E46, 1973
Mouse	Male Sprague Dawley, Schmidt Ha/ICR (n=2 or 6/group)				
Rabbit	Male New Zealand White Luenberg (n=1 or 3/group)				
Intraperitoneal/Intravenous					
Rat	Male Sprague Dawley, Charles River (n=6/group)	i.p. in aqueous solution	Up to 2033 mg/kg	No deaths within 7 days at any dose	Motor and behavioural activities unremarkable. Ref: E46, 1973
Mouse	Male Sprague Dawley, Schmidt 11a/ICR (2 or 6/group)	i.p. as 2% aqueous suspension	Up to 1000 mg/kg		
Rat	Male Charles River CD (n= 6/group)	i.v. as 5 ml/kg bw of 2% aspartame in 0.9% aqueous sodium chloride solution	100 mg	No deaths up to 72 hours post-injection	Physical, ophthalmoscopic, gross or microscopic parameters unremarkable. Phlebitis at site of implantation; kidney and endocardium inflammation. Ref: E84, 1974
Dog	Male Beagle (n=4/group)	i.v. as 5 ml injection of 0.5% aqueous sodium chloride solutions	100 mg	No deaths up to 72 hours post-injection	Physical and biochemical parameters, ECC unremarkable. Ref: E85, 1974

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6623 (b) DKP

	Strain	Route	Dose range	Reported Result	Comments and reference(s)
Oral					
Rat	Male Sprague Dawley, Charles River (n=6/group)	p.o. as 15-17.5% suspension in 1% Tween 80 (aqueous 2% suspension)	Up to 5000 mg/kg	No deaths within 7 days at any dose	Motor and behavioural activities unremarkable. Ref: E45, 1973
Mouse	Male Sprague Dawley, Schmidt Ha/ICR (n=2 or 6/group)				

Rabbit	Male New Zealand White Luenberg (n=1 or 3/group)	for low doses)			
Intraperitoneal					
Rat	Male Sprague Dawley, Charles River (n=6/group)	i.p. as 2% aqueous suspension	Up to 1562 mg/kg	No deaths within 7 days at any dose	Motor and behavioural activities unremarkable. Ref: E45, 1973

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6626 **G. TABLES ON SHORT TERM AND SUBCHRONIC TOXICITY STUDIES ON ASPARTAME AND DKP**

6627 1. Aspartame

Strain	Dose range	Duration	Reported Result	Comments and reference(s)
Oral				
“Young” purebred beagles, 7.2 – 13.2 kg (2M/2F per group)	5/125 mg/kg bw/day via gelatine capsules	8 weeks	100% survival; variable pattern of body weight loss/maintenance/gain	No consistent haematology, clinical chemistry or urinalysis findings; no consistent ocular findings; no gross pathology; no consistent effects on organ weights; no cytopathological changes. Refs: E21, 1969.
Via diet				
Charles River CD rats, 7 wk old (5/sex per group)	2000/4000/10000 mg/kg bw/day via diet; actual consumption within 10% of planned	4 weeks	100% survival; no unequivocal effect on body weight or food consumption except decreased consumption in high dose females, wks 2-3.	No adverse physical/behavioural changes; no treatment-related pathological changes except for clear, viscous fluid coating intestinal mucosa in treated rats. N.B. only examined 5 controls and all high dose animals; remaining animals were discarded. Refs: E3, 1972.
Charles River CD rats, sexually mature (10/sex per group). Group allocation by stratified randomisation	5/125 mg/kg bw/day via diet	8 weeks	100% survival; no unequivocal effect on body weight or food consumption	At sacrifice, biochemistry and pathological examination was conducted on 5 animals from each dose and the control group. No signs of systemic toxicity or pharmacological effects. No treatment related changes in haematology or urinalysis; increased terminal blood sugar at 125 mg/kg bw/day but no other significant changes in clinical chemistry. Organ weights unaffected except for increased liver/body weight ratio in high dose males. No histopathological findings except bile duct hyperplasia/ pericholangitis in all groups. Refs: E20, 1969.
Charles River CD rats, weanlings (5/sex per group)	9:100 ratio in diet (aspartame) 5:100 ratio in diet (Phe)	9 weeks	Two deaths; one control male, one aspartame-treated male. Relative food consumption comparable between groups. No treatment-related effect on terminal body weight.	No physical/behavioural abnormalities; no significant treatment related changes in haematology, clinical chemistry, urinalysis; no treatment-related changes in organ weight or pathological observations. Reduced body weight gain in treated groups, both compounds and both sexes; less marked in females than males. Refs: E4.
Ha/ICR mice, 8 wk old (5/sex per group)	Planned: 2000/4000,/10000 mg/kg bw/day via diet; Actual: 3000/5000/13000 mg/kg bw/day	4 weeks	100% survival; no unequivocal effect on body weight	The actual intake of the test compound exceeded by 25-30% in all dose groups, due to unforeseen increase in food consumption. No adverse physical/behavioural changes; no treatment-related pathological changes except for clear, viscous fluid coating intestinal mucosa in treated mice. N.B. only examined 5 controls and all high dose animals; remaining animals were discarded. Refs: E2, 1972.

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6630 2. DKP

Strain	Dose range	Duration	Reported Result	Comments
Oral				
Charles River CD rats, 11 wk old (10/sex per group)	1000 mg/kg bw/day p.o. as 5% aqueous suspension	Daily for 2 weeks	100% survival; no unequivocal effect on body weight	No physical/behavioural changes; no treatment related changes in haematology, clinical chemistry, urinalysis; no treatment-related pathological changes. Refs: E7, 1971.
Ha/ICR mice, 4 wk old (10M per group)	1000 mg/kg bw/day p.o. as 5% aqueous suspension	Daily for 2 weeks	100% survival; no unequivocal effect on body weight	No physical/behavioural changes; no treatment related changes in haematology, clinical chemistry, urinalysis; no treatment-related pathological changes. Refs: E6, 1971.
Via diet				
Charles River CD rats, 8 wk old (5/sex per group)	1000/2000/4000/6000 mg/kg bw/day via diet; actual consumption within 5% of planned	5 weeks	100% survival; reduced body weight in high dose females due to reduced food consumption.	No physical/behavioural changes; no dose-related changes in haematology, clinical chemistry, urinalysis; no dose-related changes in organ weight or treatment-related pathological changes. Evidence of colonic parasites, mild chronic bronchitis and mild chronic inflammation of liver and bile ducts. Refs: E8, 1972.

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6633 H. TABLES ON GENOTOXICITY STUDIES ON ASPARTAME, METHANOL AND DKP

6634 1. Aspartame genotoxicity studies in vitro

Test system	Test Object	Doses	Reported Result	Comments and reference(s)
<i>Salmonella typhimurium</i> Tester strains: TA1535,TA1537,TA1538 TA98, TA100	Gene mutation	[µg/plate] 10, 50, 100, 500, 1000, 5000	Negative ^{a)}	Reported identity of test compound (SC-18862; Lot no.89300); non- GLP but well conducted and reported study; deviations from current OECD TG 471: Tester strains <i>S. typhimurium</i> TA102 or <i>E. coli</i> WP2uvrA bearing AT mutation were not used. Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: E97, 1978
<i>Salmonella typhimurium</i> Tester strains: TA1535, TA1537, TA1538, TA98,TA100	Gene mutation	[µg/plate] 10, 50, 100, 500, 1000, 5000	Negative ^{a)}	Reported identity of test compound: SC-18862; Lot no.C-0096-89300; Non-GLP but well conducted and reported study; Deviations from current OECD Guideline no.471: Tester strains (TA102 or WP2uvrA) bearing AT mutation were not used. Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: E101 (1978)
<i>Salmonella typhimurium</i> Tester strains: TA1535, TA1537, TA97,TA98, TA100	Gene mutation	[µg/plate] 100, 333, 1000, 3333, 10000	Negative in TA1535, TA98, TA100 without and with S9 metabolism ^{a)} TA1537: Negative with rat liver S9; TA97: Negative without S9 or with hamster liver S9; TA97: small increases in mutant colonies with 30% rat liver S9	GLP-study; Deviation from OECD 471: Tester strains <i>S. typhimurium</i> TA102 or <i>E. coli</i> WP2uvrA bearing AT mutation were not used; TA1337 was only assayed in the presence of S9 metabolism; Study authors judged small increase in mutant colonies with 30% rat liver S9 as equivocal. Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: NTP (2003)

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Test system	Test Object	Doses	Reported Result	Comments and reference(s)
<i>Salmonella typhimurium</i> Tester strains: TA98; TA100	Gene mutation	[µg/plate] 50, 100, 250, 500, 1000, 2000	Negative ^{a)}	Major deviations from current OECD guideline no. 471: Only two tester strains used; Highest dose-level employed was lower than 5 mg/ml; Second experiment performed was identical to the first one. Overall judgement: The methods implemented were thought not to be sufficiently robust to support the results reported. Ref: Rencuzogullari <i>et al.</i> (2004)
Primary rat hepatocytes	Unscheduled DNA Synthesis (UDS)	0.1 and 5 mM	Negative	The study meets requirements of OECD Guideline (1986). Overall judgement: The methods implemented were thought to be robust to support the results reported. Ref: Jeffrey and Williams (2000)
Human lymphocyte	Chromosome aberrations (CA)	0.5, 1.0 and 2 mg/ml for 24 and 48 hours performed only in the absence of S9 metabolism	Positive ^{b)}	Major deviations from current OECD Guideline 473, 479 and 487 for all studies conducted: Culture initiation and treatment schedule inappropriate; No treatment in the presence of S9 metabolism; No measurement of pH and osmolarity values. In addition: For CA: Analyses of chromosomal aberrations performed mainly in M2 and M3 cells (Table 2); Method for scoring of aberrations do not refer to any published literature; CA do not exhibit a dose-response. For MN: Historical control range values not reported and used to accept validity of the study; MN formation concurrent with cytotoxicity (suggestive of indirect effects of aspartame); Overall judgement: The methods implemented were thought not to be sufficiently robust to support the results reported. Ref: Rencuzogullari <i>et al.</i> (2004)
	SCE		Negative ^{b)}	
	Micronuclei (MN)		Positive ^{b)}	

6636 a) With and without metabolic activation.

6637 b) Without metabolic activation.

6638 c) With metabolic activation.

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6641 2. Aspartame genotoxicity studies in vivo

Test system	Test Object	Route	Doses	Reported Result	Comments
Rat Charles River CD strain 15 males/group	Dominant lethal assay	Control (vehicle) and SC-18862: intragastric. Positive control (methyl methane sulphonate): i.p.	0 (control), 2000 mg/kg bw, two equally divided doses on single day (2 h interval)	Negative (paternal growth, maternal pregnancy rate, uterine and ovary examination data and incidence of fetal deaths)	Reported identity of test compound: SC-18862, containing 0.2% DKP (SC-19192) No Guidelines available in 1973 (since 1984: OECD 478). Study protocol followed general recommendations Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: E40 (1973)
Rat Charles River CD strain 15 males/group	Dominant lethal assay	Control (vehicle) and SC-18862: intragastric. Positive control (methyl methane sulphonate): i.p.	0 (control), 2000 mg/kg bw, two equally divided doses on single day (2 h interval)	Negative (paternal growth, maternal pregnancy rate, uterine and ovary examination data and incidence of fetal deaths)	Reported identity of test compound: SC-18862 containing 0.7% DKP (SC-19192); No Guidelines available in 1973 (since 1984: OECD 478). Study protocol followed general recommendations; Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: E41 (1973)
Rat 10 males /group	Chromosome aberration in bone marrow erythrocytes	Control (vehicle) and SC-18862: intragastric. Positive control (triethylene-melamine): i.p.	0 (control), 500, 1000, 2000 and 4000 mg/kg bw/day, given in three equally divided doses every three h, for five consecutive days	Negative	Reported identity of test compound: SC-18862, Lot#76040C, containing 0.7% DKP; GLP: no statement, but well documented report; Deviations from current OECD guideline 475: 50 spreads/rat instead of 100, but 10 instead of 5 rats. No mitotic index determination: 'The mitotic index should be determined as a measure of cytotoxicity in at least 1000 cells per animal for all treated animals (including positive controls) and untreated negative control animals'. Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: E43 (1972)
Rat 10 males/group	Chromosome aberrations in bone marrow erythrocytes and spermatogonial cells	Oral (intragastric)	0 (control), 400, 800, 1200 and 1600 mg/kg bw/day for five consecutive days	1)Negative in bone marrow erythrocytes; 2)Negative in spermatogonial cells	Reported identity of test compound: SC-18862; Lot# A3427; Major deviations from current OECD Guideline 475: Sampling time selected 24 h + 5 h; Colchicine (total 29 h). It should have been 1.5 cell cycle (12-18h); The highest dose may also be defined as a dose that produces some indication of toxicity in the bone marrow (e.g. greater than 50% reduction in mitotic index). In this study mitotic indices were not even scored; 50 metaphases per animal were scored. Dose-levels administered of test

Test system	Test Object	Route	Doses	Reported Result	Comments
					substance 0.4, 0.8, 1.2 and 1.6 g/kg bw/day are different from those reported in the conclusion of report; The second positive control employed (cyclohexylamine) did not show positive results expected. Overall judgement: The methods implemented were thought not to be sufficiently robust to support the results reported. Ref: E12 (1970)
Rat 10 males/group	Host-mediated assay	Control (vehicle) and SC-18862: oral Positive control (dimethylnitrosamine): i.p.	0 (control), 500, 1000, 2000 and 4000 mg/kg bw/day, given in three equally divided doses every three h, for five consecutive days.	Negative	Reported identity of test compound: SC-18862, Lot#76040C, containing 0.7% DKP; No Guidelines available; Study protocol followed general recommendations. Single use of G-46 Salmonella typhimurium, tester strain strongly reduce the capability to identify different classes of genotoxins. Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: E44 (1972)
Mouse 10 males/group	Host-mediated assay	Oral (intragastric)	0 (control), 1000, 2000, 4000, 8000 mg/kg bw/day three times daily at two hour intervals, for five consecutive days.	Negative	Reported identity of test compound: SC.18862 Lot#76060R; No Guidelines available; Study protocol followed general recommendations; Single use of G-46 Salmonella typhimurium, tester strain strongly reduce the capability to identify different classes of genotoxins. Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: E81 (1974)
Mouse strain C57/black 5 animals/group	Chromosomal aberrations in bone marrow erythrocytes	Oral	0 (control), 40 and 400 mg/kg bw, 5 days.	Negative	Study is poorly reported. Concurrent positive control animal group to show whether the test system was functioning correctly was not included; Concurrent negative control group was included; A minimum number of 100 metaphases per animal were scored; Dose-levels administered appear to be very low. Sampling of bone marrow cells 6 hours from last administration of test compound is not adequate for chromosomal aberration analysis. Overall judgement: The methods implemented were thought not to be sufficiently robust to support the results reported. Ref: Durnev <i>et al.</i> (1995)
Mouse 5 males/group	Chromosome aberrations in bone marrow erythrocytes	Oral (intragastric)	ASP + Acesulfame K (3.5 + 1.5) mg/kg bw ASP + Acesulfame K (35 + 15) mg/kg bw ASP + Acesulfame K (350 + 15) mg/kg bw	Negative	Study not relevant for evaluation of aspartame because aspartame was blended with acesulfame K. Ref: Mukhopadhyay <i>et al.</i> (2002)

Test system	Test Object	Route	Doses	Reported Result	Comments
Rat strain Fisher 344 5 males / group	Micronuclei in bone marrow erythrocytes	Oral (gavage)	0 (control), 500, 1000 or 2000 mg/kg bw, 3 times at 24 hr intervals.	Negative	Lot 8415-14-02 RTI; Purity: >98%; GLP-study; Deviation from OECD 475:- only male animals were used - Sampling time selected 24 h. It should have been 1.5 cell cycle (12-18h). Cytotoxicity: %PCE not greatly altered but in slight decrease (60.8 to 39%) in 1000 mg/kg group. Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: NTP (2003)
Transgenic Mouse FVB/N-TgN (v- Ha-ras)Led (Tg.AC) Hemizygous 15 males and 15 females/group	Micronuclei in peripheral blood combined with carcinogenicity	Oral (via diet)	0, 3125, 6250, 12500, 25500, 50000 ppm for 9 months Males: 490, 980, 1960, 3960, 7660 mg/kg bw Females: 550, 1100, 2260, 4420, 8180 mg/kg bw	Negative	Lot 8415-14-02 RTI;Purity: >98%; GLP-study; Deviation from OECD 474 (use of transgenic animals covered by ‘any appropriate species’): - study does not include positive control - Sampling time: authors refer to McGregor et al. 1990 but did not specify the actual time point. No evidence of carcinogenic activity in TgAc haplo insufficient male or female mice. Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: NTP (2003)
Transgenic Mouse B6.129- Trp53 ^{tm1Brd} (N5) haploinsufficient 15 males and 15 females/group	Micronuclei in peripheral blood combined with carcinogenicity	Oral (via diet)	0, 3125, 6250, 12500, 25500, 50000 ppm for 9 months Males: 490, 970, 1860, 3800, 7280 mg/kg bw Females: 630, 1210, 2490, 5020, 9620 mg/kg bw	3125 up to 25500 mg/kg diet group: males and females negative 50000 mg/kg diet group: females judged to be positive based on a significantly increased frequency (x 2.3) of micronucleated erythrocytes	Lot 8415-14-02 RTI; Purity: >98%; GLP-study; Deviation from OECD 474 (use of transgenic animals covered by ‘any appropriate species’): - study does not include positive control - Sampling time: authors refer to McGregor et al. 1990 but did not specify the actual time point. No evidence of carcinogenic activity in TgAc haploinsufficient male or female mice. Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: NTP (2003)
Transgenic Mouse Cdkn2a deficient 15 males and	Micronuclei in peripheral blood	Oral (via diet)	0, 3125, 6250, 12500, 25500, 50000 ppm for 9 months.	Negative	Lot 8415-14-02 RTI; Purity: >98%; GLP-study; Deviation from OECD 474 (use of transgenic animals covered by ‘any appropriate species’): study does not include positive control; sampling time: authors refer to McGregor <i>et al.</i> 1990 but did not specify the actual time point. No evidence of carcinogenic activity in TgAc haploinsufficient male or female mice (no evidence: no chemical- related increases in malignant or benign neoplasms.

Test system	Test Object	Route	Doses	Reported Result	Comments
15 females/group					Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: NTP (2003)
Mouse 4 males/group	Comet assay in Stomach, colon, liver, kidney, bladder, lung, brain, bone marrow	Oral (intragastric)	2000 mg/kg bw	Negative in all organs assayed	Deviations from minimum requirements: 1) Four animals used; 2) Only one dose-level employed (2000 mg/kg bw); 3) Two slides analysed (50 nuclei/slide); 4) Manual scoring: DNA migration was evaluated as the difference between length of the tail and diameter of the nucleus. 5) Isolated nuclei instead of whole cells were employed; 6) Cell viability not reported. Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: Sasaki <i>et al.</i> (2002)
Mouse 4 males/group	Comet assay in bone marrow cells	Oral (intragastric)	0 (control), 7, 14, 28 and 35 mg/kg bw	Positive at higher dose-level	The study is generally poorly reported; Deviations from recommended protocol: 1) Insufficient number of cells scored (total of 50 cells/animal); 2) Historical control values not reported/ Overall judgement: The methods implemented were thought not to be sufficiently robust to support the results reported. Ref: Bandyopadhyay <i>et al.</i> (2008)
Mouse (Number of animals not clearly reported)	Micronuclei in bone marrow erythrocytes	Oral (intragastric)	0 (control), 250, 455, 500 and 1000 mg/kg bw	Positive	The study is generally poorly reported. Deviations from OECD guideline: 1) Number of animals employed is not reported; 2) The PCE/NCE ratio is described as 'changed' but no data are given 3) No criteria for selection of higher dose-level. 4) no historical control data. Ref: Kamath <i>et al.</i> (2010)
	Micronuclei in peripheral blood			Positive	
	Chromosome aberrations in bone marrow erythrocytes			Positive	Deviations from OECD guideline: 1) Number of animals employed not clearly reported; 2) No mitotic indices scored; 3) Treatment with colcemid too short (1.5 hours instead of 3-5 hours); 4) No criteria for selection of higher dose-level. Overall judgement: The methods implemented were thought not to be sufficiently robust to support the results reported.
Mouse (5 males/group)	Chromosome aberrations in bone marrow erythrocytes	Oral (intragastric)	0 (control), 3.5, 35 and 350 mg/kg bw	Positive	Deviations from OECD guideline: 1) insufficient number of cells scored; 2) Any supplementary information on cytotoxicity relevant for CA and SCE-analysis in the present experiment not given 3) No positive control: No Guidelines available. Number of cells scored (30 cells/animal for SCE) insufficient for statistical analysis.

Test system	Test Object	Route	Doses	Reported Result	Comments
	SCE in bone marrow erythrocytes			Negative	Overall judgement: The methods implemented were thought not to be sufficiently robust to support the results reported. Ref. Al Suhaibani <i>et al.</i> (2010)

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6643 3. Methanol genotoxicity studies in vitro

Test system	Test Object	Doses	Reported Result	Comments
<i>Salmonella typhimurium</i> Tester strains: TA1535, TA1537, TA1538, TA 98, TA100	Gene mutation	No data. Various dilutions performed by geometric ratio starting from solubility or toxicity limit.	Negative ^{a)}	Not assignable Survey of the capacity of 135 compounds incl. methanol. Concentration of test compound not given. Ref: DeFlora <i>et al.</i> (1984)
<i>E. coli</i> Tester strains: WP2; WP2 (<i>uvrA</i> -, <i>polA</i> -); CM871 <i>uvrA</i> - , <i>recA</i> -, <i>lexA</i> -)	DNA repair	No data. Various dilutions performed by geometric ratio starting from solubility or toxicity limit.	a) Microtiter plate: negative ^{a)} b) 2-h preincubation: without S9: negative; with S9: 1.5-2 fold increase+ c) spot-test: negative	Not assignable Survey of the capacity of 135 compounds incl. methanol Concentration of test compound not given. Statement by MAK (1999): 'As it was conceivable that the weak positive results obtained with methanol were caused by impurities, the authors classify methanol as an uncertain or questionable mutagen' Ref: DeFlora <i>et al.</i> (1984)
<i>Neurospora Crassa</i>	Chromosome aberrations	Not reported	Negative	A study which included 57 compounds; No dose-levels for methanol reported; Test system not currently employed to detect aneugens. Overall judgement: The methods implemented were thought not to be sufficiently robust to support the results reported. Ref: Griffiths (1981)
<i>Salmonella typhimurium</i> Tester strains: TA1535, TA1537 TA1538, TA98, TA100	Gene mutation	No data	Negative (not described: with or without S9)	Not assignable Not described: with or without S9as well as MeOH concentration. MeOH listed as one of 45 chemicals that gave negative results. No conclusion can be drawn from the publication. Ref: Simmon <i>et al.</i> (1977)
<i>Salmonella typhimurium</i> Tester strains: TA1535, TA1537 TA1538, TA 98 TA100	Gene mutation	5 doses up to 3.6 mg/plate	Negative ^{a)}	Not assignable Screening study on genotoxicity of 31 chemicals. Bacterial reverse mutation assay followed standard protocol of that time. No detailed results on methanol are reported. Deviations from current OECD Guideline 471: - TA102, <i>E. coli</i> not included in the study - maximal concentration tested (3.6 mg/plate) lower than recommended - range, but not actual concentration tested given. Ref: Gocke <i>et al.</i> (1981)

Test system	Test Object	Doses	Reported Result	Comments
<i>Salmonella typhimurium</i> Tester strains: TA1535, TA1537 TA1538, TA 98 TA100	Gene mutation	2.5 mM/plate (733 µg/plate)	Negative ^{a)}	DeFlora <i>et al.</i> 1981 cited from MAK-Liste - TA 102, <i>E. coli</i> not included. Ref: DeFlora <i>et al.</i> (1981)
<i>Salmonella typhimurium</i> Tester strains: TA1535, TA1537 TA1538, TA 98 TA100	Gene mutation	[µg/plate] 5, 10, 50, 100, 500, 1000, 5000	Negative ^{a)}	Both in the absence and presence of rat liver S9 metabolism. Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: Shimizu <i>et al.</i> (1985)
<i>E. coli</i> Tester strain: WP2 uvrA <i>Schizosaccharomyces pombe ade6-60/rad10-198,h⁻</i> (P1 strain)	Gene mutation	5%	-S10/+S10 mouse: Negative	Non-GLP study; Material and methods described in sufficient details; Maximum concentration for mutagenicity test was based upon cytotoxicity at concentrations of 0.5, 2, 5 and 10% (v/v). Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: Abbondandolo <i>et al.</i> (1980)
<i>Aspergillus nidulans</i> (diploid strain P1)	Chromosome aberrations	5.2, 5.6, 6, 7 % (v/v)	Positive Cross over: negative	Non-GLP study; Material and methods described in sufficient details; Exposure to test compound during early germination; Aneuploids (non-disjunctional diploids and haploids) at toxic concentration 5.6 and 6% MeOH (survival: 19 and 10%, respectively). Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: Crebelli <i>et al.</i> (1989)
<i>Salmonella typhimurium</i> Tester strains: TA1535, TA1537 TA1538, TA 98 TA100	Gene mutation (Pre-incubation) Absence and presence of S9	[µg/plate] 10, 50, 100, 500, 1000, 5000	Negative ^{a)}	No GLP-statement; Deviation from OECD 471: - no historical control data Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: NEDO (1987)
<i>E. coli</i> Tester strain: WP2 uvrA				

Test system	Test Object	Doses	Reported Result	Comments
Chinese Hamster lung fibroblasts (V79 cells)	Gene Mutation (azaguanine, 6-thioguanine, ouabain)	15.8, 31.7, 47.4, 63.3 mg/ml exposure: 3 h	Negative ^{a)}	63.3 mg/ml inhibit cell colony formation by approximately 70%; Mutant selection after 6 days; No GLP-statement; Deviation from OECD 476: - no historical data - concentration of methanol exceeded the maximum concentration recommended in <i>in vitro</i> studies (i.e. 5 mg/mL); Overall judgement: The methods implemented were thought not to be sufficiently robust to support the results reported. Ref: NEDO (1987)
	Chromosomal aberration	7.1, 14.3, 28.5 mg/ml for 2 days	Negative ^{a)}	28.5 mg/ml inhibit cell growth by approximately 50%; Chromosome preparation at 6, 24, 48 h after treatment; 200 cells/dose-level scored; No GLP-statement; Deviations from OECD 473: - treatment schedule and sampling times; - no cytotoxicity measured (mitotic index, cell counts or degree of confluence); - no historical data - concentration of methanol exceeded the maximum concentration recommended in <i>in vitro</i> studies (i.e. 5 mg/mL); Overall judgement: The methods implemented were thought not to be sufficiently robust to support the results reported. Ref: NEDO (1987)
Chinese Hamster lung fibroblasts (V79 cells)	SCE	7.1, 14.3, 28.5 mg/ml	7.1, 14.3 mg/ml negative ^{a)} 28.5 mg/ml -S9: positive +S9: negative	Highest concentration (28.5 mg/ml) SCE-incidence of 9.41 ± 0.416 significantly greater than controls 6.42 ± 0.227 [mean \pm SE per 100 cells]; Sampling: 24-26 after treatment; 100 cells/dose level scored; No GLP-statement; Deviation from OECD 479: - no duplicate cultures - no cytotoxicity measured (mitotic index, cell counts or degree of confluence) - concentration of methanol exceeded the maximum concentration recommended in <i>in vitro</i> studies (i.e. 5 mg/mL); Overall judgement: The methods implemented were thought not to be sufficiently robust to support the results reported. Ref: NEDO (1987)
<i>E. coli</i> (SA500)	(Gene mutation) forward mutation	23-31%	Negative	Study focussed on ethanol: Methanol was included for comparison. Methanol concentrations above 23 % were cytotoxic, reducing survival of the cells to less than 40 %. A 23 % methanol solution produced no mutagenic effects; cell survival was 53 %. However, assay performed with one <i>E. coli</i> strain <i>E. coli</i> (SA 500) as well as in the absence of metabolic activation system only. Overall judgement: The methods implemented were thought not to be sufficiently robust to support the results reported. Ref: Hayes et al. (1990)
MCF-7 and HepG2 cells	Activation of the p53R2 gene		Negative	Assay based on chemical activation of the p53R2 gene as assessed by the incorporation of a p53R2-dependent luciferase reporter gene into human cells (MCF-7 and HepG2). The Panel considered this study 'reliable' but not suitable for the present evaluation, because the endpoint used was not relevant for the mechanism of action of ionizing radiation or non-radiomimetic

Test system	Test Object	Doses	Reported Result	Comments
				chemical compounds Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: Ohno <i>et al.</i> (2005)
Mouse lymphoma L5178Y(Tk+/Tk-)	Gene mutation	5-50 µl/ml (approx. 3.95-39.5 mg/ml)	Negative b) Positive c)	Significant increases in mutation frequency were observed at increased concentrations of S9. However, the lowest concentration tested (5 µl/ml) exceeded in terms of molarity (13.4 M) the maximum concentration recommended in OECD guideline 476 (5 mg/ml, 5 µl/ml, or 0.01 M, whichever is the lowest). Therefore, indirect effects due to non-physiological culture conditions cannot be excluded. Overall judgement: The methods implemented were thought not to be sufficiently robust to support the results reported. Ref: McGregor <i>et al.</i> 1985, 1988)
<i>Saccharomyces cerevisiae</i> (ATCC26422)	Gene mutation	2-8%	-S9: negative +S9: Not performed	Methanol (2-8%) was assessed for induction of gene mutations in <i>Saccharomyces cerevisiae</i> ATCC26422 as a control culture within a study aimed at elucidating the effects of ethidium bromide. Methanol did not induce gene mutations, but was tested without metabolic activation system only. The Panel considered the study as 'not reliable' in view of assessing the genotoxic potential of methanol. Overall judgement: The methods implemented were thought not to be sufficiently robust to support the results reported. Ref: Hamada <i>et al.</i> (1988)
<i>E. coli</i> WP2 (λ)	Prophage-induction	0.15; 0.31; 0.62; 1.25; 2.5; 5%	Negative ^{a)}	Materials and methods described in sufficient details; In two independent experiments MeOH was tested up to toxic concentrations. Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: De Marini <i>et al.</i> (1991)
Chinese hamster (V79 cells)	Sister chromatid exchange (SCE) and micronucleus	50 µl/ml	Negative	Treatment: single dose-level (50 µl/ml) which was 10 times greater than the maximum recommended dose-level to be used in <i>in vitro</i> studies (e.g. 5 µl/ml) to keep culture physiological conditions; Assayed in the absence of S9 only; Treatment time: 48 hours, which was exceedingly long compared to 1.5-2 cell cycle length recommended by the OECD guideline no. 487. Overall judgement: The methods implemented were thought not to be sufficiently robust to support the results reported. Ref: Lasne <i>et al.</i> (1984)

6644 a) With and without metabolic activation.
6645 b) Without metabolic activation.
6646 c) With metabolic activation.
6647

6648 4. Methanol genotoxicity studies in vivo

Test system	Test Object	Route	Doses	Reported Result	Comments
Mouse (Pregnant CD-1 Females)	Micronuclei in peripheral blood reticulocytes	Oral (intra-gastric)	2500 mg/kg Twice a day From GD 6 to GD 10	Negative both in blood of foetuses or mothers	Only one dose-level assayed; Blood samples were collected eight days after last administration of compound but normal procedure indicate sampling not later than 72 hours; The frequency of MN in maternal and fetal reticulocytes was similar among the groups; the frequency of MN was not associated with either maternal or fetal hepatic folate concentrations. Furthermore, no significant difference in the frequency of MN in reticulocytes of fetuses that were affected by malformations compared to fetuses without malformations. Overall judgement: The methods implemented were thought not to be sufficiently robust to support the results reported. Ref: Fu <i>et al.</i> (1996)
Mouse ICR strain 6 males/group	Micronuclei in bone marrow erythrocytes	Oral	1050, 2110, 4210, and 8410 mg/kg bw, single dose	Negative	GLP-study; Deviation from OECD 475: - only male animals were used - only one sampling time selected Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: NEDO 1987
Rats	Hydroxymethyl DNA adducts	Oral	500 mg/kg per day for 5 days (stable isotope labelled methanol)	Labelled DNA adducts lower than the number of endogenous hydroxymethyl DNA adducts in all tissues	Concerns about the lower number of exogenous hydroxymethyl DNA adducts measured in the analysed tissues of rats following administration of [¹³ CD ₄]-methanol at 500 mg/kg bw for 5 days compared to the number of endogenous hydroxymethyl DNA adducts. The isotope effect factor employed was the mean of several widely varied published values. Furthermore, the measurements of N-hydroxymethyl-dG and dA adducts was not direct but required an <i>in vitro</i> reduction

Test system	Test Object	Route	Doses	Reported Result	Comments
					<p>process with NaCNBH₃ to the corresponding N-methyl derivative. The measurements of endogenous N-hydroxymethyl-dA adducts in the untreated animal group and in methanol treated group at 500 mg/kg bw for 5 days were rather variable</p> <p>Overall judgement: The methods implemented were thought not to be sufficiently robust to support the results reported. Ref: Lu <i>et al.</i> 2012</p>
Drosophila melanogaster wild type and Basc strains	Sex-linked lethal mutations		1000 mM	Negative	Ref: Gocke <i>et al.</i> 1981
Mouse	Chromosomal aberrations	Oral	1000 mg/kg	Positive	<p>Not assignable</p> <p>Increased the incidence of chromosomal aberrations (particularly aneuploidy and exchanges and the micronuclei of polychromatic erythrocytes). Results of the study were available as an abstract only. Ref: Pereira <i>et al.</i> 1982</p>
<p>Mouse</p> <p>B6C3F1 strain</p> <p>15 males/group</p>		Oral (gavage)		<p>Increase in structural chromosome aberrations [exchanges (Robertsonian translocations) and aneuploidy; no breaks]</p>	<p>Deviations from current OECD475: - one dose only - one time point only - mitotic indices not determined. Significant increases in chromosome aberrations [exchanges (Robertsonian translocation), aneuploidy] in male B6C3F1 were reported. However, concurrent vehicle control showed 10 exchanges out 665 cells scored and chromatid and chromosome exchanges are not expected in healthy animals.</p> <p>Overall judgement: The methods implemented were thought not to be sufficiently robust to support the results</p>

Test system	Test Object	Route	Doses	Reported Result	Comments
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reported. Ref: Ward *et al.* 1983

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6651 5. In vivo studies by other routes of exposure (inhalation and intraperitoneal administration)

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Test system	Test Object	Route	Doses	Reported Result	Comments
Mouse C57black/6J 10 males/group	Micronuclei SCE	Inhalation	0, 800, or 4000 ppm (0, 1048, and 5242 mg/m ³) methanol, 6 hours/day, for 5 days	Endpoints: 1. blood cells: micronuclei (MN) 2. excised lung cells: Chromatid Exchanges (SCEs), Sister chromosomal aberrations (CA) and micronuclei (MN), 3. excised testicular germ: synaptonemal damage. All endpoints studied gave negative results	Inhalation route of administration not relevant for evaluation of aspartame. However, the study may be used for weight-of-evidence approach. Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: Campbell <i>et al.</i> (1991)
Mouse male CD-1			1 x 2000 mg/kg bw		Limit dose of 2000 mg/kg bw MeOH based on guidelines established for the comet assay developed in accordance with the <i>in vivo</i> genetic toxicology guidelines of (OECD. Valid study but because of i.p. route of administration study considered not relevant for present evaluation of aspartame. However, the study may be used for weight-of-evidence approach. Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: McCallum <i>et al.</i> (2011a; 2011b)
Mouse male CD-1			Chronic 15 x 2000 mg/kg (one dose per day)		
Mouse male CD-1 mice OGG1 (-/-)	DNA damage (8-oxodG)	i.p.	1 x 2000 mg/kg bw	No increase of 8-oxodG at 6 and 24 h in bone marrow, spleen lung, kidney of any species	
Rabbit male New Zealand white (NZW)			1 x 2000 mg/kg bw		
Monkey male(Macaca fascicularis)			1 x 2000 mg/kg bw		
Mouse Swiss-Webster 10 males/group	Micronuclei	Intraperitoneal	300, 600, 1200 or 2500 mg/kg body weight on 4 consecutive days	Negative	Ref: US EPA 1991
Mouse NMRI strain 2 males and 2 females/group	Micronuclei in Bone marrow erythrocytes	Single intraperitoneal injection	1920, 3200, 4480 MeOH mg/kg bw	Negative	Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: Gocke <i>et al.</i> 1981

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6654 6. DKP genotoxicity studies in vitro

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Test system	Test Object	Doses	Reported Result	Comments
<i>Salmonella typhimurium</i> Tester strains TA1535, TA98 TA1538, TA98 TA100	Gene mutation (plate incorporation with and without metabolic activation)	[µg/plate] 10 up to 5000	Negative	Reported identity of test compound: SC19192; Lot# TJT-12-32; Deviations from current OECD Guideline: 1) Tester strains (TA102 or WP2uvrA) bearing AT mutation were not used; 2) Second experiment performed was identical to the first performed. Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: E98 (1978)
<i>Salmonella typhimurium</i> Tester strains TA1535, TA1537, TA1538, TA98 and TA100	Gene mutation (plate incorporation with and without metabolic activation)	[µg/plate] 50 up to 10.000	Negative	SC-19192, Lot TJT- 12-32. It meets requirements of previous version of OECD Guideline; Deviations from current OECD Guideline no 471: Tester strains <i>S. typhimurium</i> TA102 or <i>E. coli</i> WP2uvrA bearing AT mutation were not used; Second experiment performed was identical to the first performed. Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: E106 (1978)

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6658 7. DKP genotoxicity studies in vivo

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Test system	Test Object	Route	Doses	Reported Result	Comments
Rat 10 males /group	Chromosome aberrations in bone marrow erythrocytes	Oral (intragastric) suspension in 10% tween-80/water)	0 (control), 250, 500, 1000 and 2000 mg/kg bw/day for 5 consecutive days, given in three equally divided daily doses	Negative	Reported identity of test compound: SC-19192; Lot# 3R-A-7273; Deviations from current OECD Guideline 475: Sampling time selected 24 h; The highest dose may also be defined as a dose that produces some indication of toxicity in the bone marrow (e.g. greater than 50% reduction in mitotic index). In this study mitotic indices were not even scored;50 metaphases per animal were scored; Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: E30 (1972)
Rat 10 males/group	Host-mediated assay	Oral (intragastric)	0 (control), 250, 500, 1000, 2000 mg/kg bw/day, three equally divided doses for five consecutive days. 30 minutes after the final dose, the animals were inoculated with <i>Salmonella typhimurium</i> , G-46, by intraperitoneal injection. Three hours later the bacteria were recovered and the peritoneal washing was evaluated for the presence of mutants.	Negative	Reported identity of test compound: SC-19192; Lot# 3R-A-7273; No Guidelines available; Study protocol followed general recommendations; Single use of G-46 <i>Salmonella typhimurium</i> , tester strain strongly reduce the capability to identify different classes of genotoxins. Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: E31 (1972)

Test system	Test Object	Route	Doses	Reported Result	Comments
Mouse Ha/ICR random bred Swiss 10 males/group	Host-mediated assay	Oral	0 (control), 1000, 2000, 4000, 8000 mg/kg bw/day, given in three equally divided doses at two hr intervals for five consecutive days <u>low and mid-dose</u> : 10% DKP suspension (1% aqueous Tween-80) <u>high and very high</u> dose: 15% DKP suspension (1% aqueous Tween-80).	Negative	Reported identity of test compound: SC-19192; Lot#6-R); Approximately 30 min. after the final dose, the animals were inoculated (i.p) with Salmonella typhimurium (G-46), intraperitoneally. Three hours later the bacteria were recovered and the peritoneal washing was evaluated for the presence of mutants. 2000 mg/kg bw/day: 1 of 10 animals died (compound in lung). 4000 mg/kg bw/day: 2 of 10 animals died: one animal with diarrhoea, dehydration, other animal blood clot (unknown cause). 8000 mg/kg bw/day: diarrhoea, dehydration, 70% mortality. 1000 mg/kg bw/day: discrepancy between number of survivors given in tab. 1 (n=10) and results shown in tab. 2,3 (n=7). Furthermore, this group differed significant from controls (tab. 2,3). Both points are not addressed by the authors. Overall judgement: The methods implemented were thought not to be sufficiently robust to support the results reported. Ref: E82 (1974)
Rat	Dominant lethal assay	Oral (intra gastric)	1000 mg/kg bw/day given in two equally divided doses on single day.	Negative (paternal growth, maternal pregnancy rate, corpora lutea and implantation sites). Implantation sites were sub-classified as: 1) Viable fetal swelling; 2) Early fetal deaths; 3) Late fetal deaths.	Reported identity of test compound: SC-19192; Lot# A6906; No Guidelines available; Study protocol followed general recommendations; Overall judgement: The methods implemented were thought to be sufficiently robust to support the results reported. Ref: E42 (1973)

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6663 I. TABLES ON REPRODUCTIVE AND DEVELOPMENTAL TOXICITY STUDIES ON ASPARTAME

Species	Endpoint	Doses tested (mg/kg bw/day)	Animals per group	NOAEL (mg/kg bw/day)	Observed adverse effects at higher dose(s) in pups	Observed adverse effects in mothers (dose)	Comments
Mouse	Embryotoxicity and teratogenicity (Segment 2)	0, 1000 (1400) ^a , 2000 (2700), 4000 (5700) (diet)	F:36,36,36,36	4000	N/A ^b	None	Pregnant animals: 27,25,27,21. Study E89, 1975
Rat	Embryotoxicity and teratogenicity (Segment 2)	0, 2000 (2000), 4000 (4100) (diet)	F:30,30,30	4000 (4100)	N/A	Decreased feed consumption (high dose)	Pregnant animals: 24,26,23. Study c E5, 1970
Rat	Perinatal and postnatal development (Segment 3)	0, 2000 (1800), 4000 (3600) (diet)	M/F:20,20,20	2000 (1800)	Transient effects on renal tubuli that were not observed in an extra group of pups examined at or shortly after weaning	None	This study is a evaluation of F2A pups of study E11 on PP 5,15, 21. Study E9, 1972
Rat	Reproductive performance and development (Segment 1)	0, 2000 (2400), 4000 (4900) (diet)	F: 48,30,30 M:14,14,14	4000 (4900)	N/A	Mild but statistically significant decrease in bw during gestation but feed consumption unaffected (high dose)	Exposure: M, 64 days PM ^d ; F: 14 days PM, gestation, lactation; half F sacrificed GD 13, half allowed to litter. Study E10, 1972
Rat	Two-generation reproductive study	0, 2000 (1800), 4000 (3700) (diet)	P ₁ : F: 24,24,24 M: 12,12,12 P ₂ : F: 19,20,20 M: 10,10,10	2000 (1800)	BW suppression and smaller size of pups at weaning (statistically significant)	None	Study E11, 1971
Rat	Perinatal and postnatal development (Segment 3)	0, 2000 (2000), 4000 (4000) (diet)	F: 30,30,30	2000 (2000)	Number of viable pups per litter at birth and at weaning decreased (statistically significant); no effect	Decreased bw at GD 21 (low and high dose) and PP day 21 (high dose)	GD 14- lactation d21. Study E39, 1973

Species	Endpoint	Doses tested (mg/kg bw/day)	Animals per group	NOAEL (mg/kg bw/day)	Observed effects at higher dose(s) in pups	adverse effects at higher dose(s) in mothers (dose)	Observed adverse effects in mothers (dose)	Comments
					on bw.			
Rat	Perinatal and postnatal development (Segment 3)	0, 2000 (2500), 4000 (4400) (diet)	F:24,24,24	2000 (2500)	BW suppression of pups at birth (statistically significant) and weaning (not significant). Pup number increased but survival significantly decreased. Incomplete eyelid opening in 5/164 pups and lens opacity in 2/164 pups.	Decreased bw during lactation		GD 14- lactation d21. Study E47, 1973
Rat	Perinatal and postnatal development (Segment 3)	0, 2000 (1800), 4000 (4000) (diet)	F: 30,30,30	2000 (1800)	BW suppression of pups at birth (statistically significant) and weaning (not significant). Pup survival significantly decreased. Incomplete eyelid opening in 2/79 pups.	Decreased feed intake at PP day 21 (high dose) and reduced bw at PP day 21 and during lactation (high dose).		GD 14- lactation d21. Study E48, 1973
Rabbit	Embryotoxicity and teratogenicity (Segment 2)	0, 2000 (1880), 4000 (1870) (diet)	F: 16,16,16	2000 (1880)	Serious congenital anomalies in the "high" dose group: Cleft palate, microstomia, open eyes, digital malformations; decreased fetal weight (by 25%).	Severe depression (>50%) in feed consumption		GD 6-19(morning); pregnant animals: P:14,16,16; aborted: 2,1,3. Study E54, 1974
Rabbit	Embryotoxicity and	0, 1000 (840), 2000	F: 14,14,	2000	N/A	Significant depression of		GD 6-19(morning);

Species	Endpoint	Doses tested (mg/kg bw/day)	Animals per group	NOAEL (mg/kg bw/day)	Observed effects at higher dose(s) in pups	Observed adverse effects in mothers (dose)	Observed adverse effects	Comments
	teratogenicity (Segment 2)	(1450), 4000 (1260) (diet)	14,14				feed consumption (35%) in high dose. Abortions (17%) in the high dose group.	pregnant animal: 12,14,14,13 Deaths: 0,1,1,0 Abortions: 0,0,0,2 Premature deliveries: 1,0,0,0. Study E53, 1973 Due to insufficient amount of test compound not all animals were fed the correct diets. Mid dose decreased number of fetuses
Rabbit	Embryotoxicity and teratogenicity (Segment 2)	0, 2000 (670), 3000 (1350), 4000 (1160) (diet)	F:15,15,15,1 5	4000 (1160)	N/A		Feed consumption decreased by up to 29% in the high dose group.	GD 6-19(morning); pregnant animals: 12,13,10, 12 Deaths: 1,0,0,0,0 Abortions: 2,2,1,0 Premature deliveries: 1,1,0,0. Study E55, 1973
Rabbit	Embryotoxicity and teratogenicity (Segment 2)	0, 1000 (1100), 2000 (1900) (diet)	F:30,30,30	2000	N/A	None		GD 6-19(morning); pregnant animals: 26,23,25 Deaths: 0,1,3 Abortions: 0,0,0 Premature deliveries: 1,1,1 Control group was pair-fed; High dose 3 fetuses of one litter open eye (considered by the authors as minor malformations). Study E62, 1973
Rabbit	Embryotoxicity and teratogenicity (Segment 2)	0, 1400 (1380), 2400 (2360) (diet)	F: 26, 26, 26	2400	N/A ^b			Study E63, 1973

Species	Endpoint	Doses tested (mg/kg bw/day)		Animals per group	NOAEL (mg/kg bw/day)	Observed adverse effects at higher dose(s) in pups	Observed adverse effects in mothers (dose)	Comments
Rabbit	Embryotoxicity and teratogenicity (Segment 2)	0, (intra-gastric)	2000	F:12,36	2000	N/A ^c	Study confounded by poor health and gastric intubation technique issues; high maternal mortality; depression of feed consumption by 40% compensated by pair-feeding of controls.	GD 6-18; pregnant animals: 5,11 Deaths: 4,9 Abortions: 1,2 Feed consumption: control 21.2g/kg; treated group 34.6 g/kg. Very low pregnancy rate. Authors mention infectious pulmonary disease and dosing errors. No malformations. Study E51, 1973
Rabbit	Embryotoxicity and teratogenicity (Segment 2)	0, (intra-gastric)	2000	F:24,72	2000	N/A	Study confounded by poor health and gastric intubation technique issues; pregnancy rate decreased in high dose group; depression of feed consumption by up to 62% compensated by pair-feeding of controls.	GD 6-18; NP animals: 3,25 Deaths: 1(NP), 6 (P) + 6 (NP) Abortions: 3,0 Controls had lower bw and fetal weight; increased no. of resorptions: 0.7 vs 1.6 (not significant); historical control 0-1.1. Study E52, 1973
Rabbit	Embryotoxicity and teratogenicity (Segment 2)	0, 750, (intra-gastric)	2000	F: 32,37,95	750	Major malformations in 7 fetuses of high dose group	Study confounded by poor health and gastric intubation technique issues; pregnancy rate decreased in high dose group; depression of feed consumption by up to 37% compensated by pair-feeding of controls. Increased no. of resorptions 0.9 vs 0.5 in	GD 6-18; pregnant animals: 27,25,61 Deaths: 3,5,9 Abortions: 3,0,0 Premature deliveries:1,1,0 Implantation scars at C-section: 5,1,16. Study E79, 1974

Species	Endpoint	Doses tested (mg/kg bw/day)	Animals per group	NOAEL (mg/kg bw/day)	Observed adverse effects at higher dose(s) in pups	Observed adverse effects in mothers (dose)	Comments
						controls and decreased no. of live fetuses (high dose)	
Rabbit	Embryotoxicity and teratogenicity (Segment 2)	0, 500, 1000, 2000 (intragastric)	F: 50,50,50 (52),50	1000	Decreased pup weight and skeletal abnormalities at the high dose only.	A 67% decrease in feed intake and up to a 15% bw decrease (GD 22) at the high dose; high incidence of abortions in the high dose group that correlated with the severity in feed intake decrease in individual animals.	GD 6-18; pregnant animals: 42,46,48,37 Deaths: 4,4,1,4. NP: 8,4,4,13 Abortions: 0,2,0,24 Premature deliveries: 3,0,1,0 High number of abortions; Increased no. of resorptions (not significant). Study E90, 1975

6664 ^a In brackets, dose level consumed
6665 ^b N/A, Not applicable, (NOAEL was at the highest dose tested)
6666 ^c Study based on small number of animals.
6667 ^d Abbreviations: PM, pre-mating; F, female; M, male; GD, gestation day; G, gestation; L, lactation, P, pregnant; NP, non-pregnant

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6670 **J. TABLES ON HUMAN STUDIES (UNPUBLISHED AND PUBLISHED) REPORTING PHENYLALANINE AND ASPARTATE LEVELS AFTER ASPARTAME DOSING**

Subjects/Study	Doses	Duration	Plasma Phe	Plasma Aspartate	Comments
HEALTHY					
SINGLE DOSE					
One 12 year-old healthy male and one 15-year old healthy female	Loading dose of 34 mg/kg bw aspartame; 2 weeks later: equivalent amount of Phe (19 mg Phe/kg).		No significant differences reported.	NR	E66, 1973. Tolerance of loading doses of aspartame by normal adolescent.
6 healthy males and 6 healthy females /cross over	Loading dose of 34 mg/kg bw aspartame; equivalent amount of aspartate (13 mg/kg).		Increased from 5-6 umol/dl (normal fasting level) to 120 ± 30 umol/L (after aspartame load), within normal postprandial range. Returned rapidly to normal.	Unchanged (2-8 umol/L).	
6 healthy lactating females/cross over	Loading dose of 50 mg/kg bw either aspartame or lactose.		Increased to peak value of 162 ± 49 umol/L (after aspartame load only), only slightly higher than those noted postprandially (120 ± 30 umol/L).	Unchanged	Small increases in breast milk aspartate and Phe to postprandial range. E93, 1977. Effect of aspartame loading upon plasma and erythrocyte free amino acid levels in normal adult subjects
3 healthy males and three females/cross over	Loading dose of 100 mg/kg bw aspartame either dissolved in cold orange juice or as a slurry.		Increased levels to about 200-260 umol/L with aspartame in solution. Aspartame in a slurry: peak at about 510 umol/L after 1 hour (2 subjects), at over 300 umol/L after 2 hours (2 other subjects), at about 180 umol/L after 3 hours (2 other subjects).	Little/no effects with aspartame in solution. Increased with aspartame as a slurry in two out of the 6 subjects: peak of 36 and 58 umol/L; mean peak about 15 ± 18 umol/L (4 subjects no changes).	
3 healthy males and					

Subjects/Study	Doses	Duration	Plasma Phe	Plasma Aspartate	Comments
three females	Loading dose of 150 mg/kg bw aspartame		Increased level, peak at about 351 ± 113 umol/L	Very small changes, peak at 10 ± 7 umol/L	
3 healthy males and three females	Loading dose of 200 mg/kg bw aspartame		Increased level, peak at about 487 ± 15 umol/L after about 2 hours	Very small changes, peak at 7 ± 3.8 umol/L at 90 min	
3 healthy males and 3 females/cross over	High protein meal containing aspartame plus MSG at 34 mg/kg bw		Slightly increased, peak around 80-90 umol/dL after 3-4 hours (only slightly higher than those noted postprandially, 120 ± 30 umol/L)	Unchanged.	The addition of aspartame into the meal increased Phe content from 43 to 64 mg/kg. E95, 1977. Metabolic studies of aspartame and monosodium glutamate (MSG) ingested as a meal component.
12 healthy infants (8-12 months)	Loading dose of 34 mg/kg bw aspartame		Slightly increased, from 63 ± 12 umol/L (zero time) to 97 ± 27 umol/L after 0.5 hours.	Unchanged or decreased from level observed at zero time (51 ± 33 umol/L), but higher compared to adults even at zero time (3 umol/L).	E107, 1977. Effect of aspartame loading upon plasma and erythrocyts, free amino acid levels and blood methanol levels in normal one-year old children.
	Loading dose of 50 mg/kg bw aspartame		Increased after aspartame ingestion, from 57 ± 5 umol/L (zero time) to 116 ± 44 umol/L after 1 hour.	Unchanged from level observed at zero time (30.3 ± 15 umol/L) but higher compared to adults even at zero time (4.2 ± 3.3 umol/L).	
8 healthy infant s (8-12 months)	Loading dose of 100 mg/kg bw aspartame		Increased after aspartame ingestion, from 48 ± 8 umol/L (zero time) to 214 ± 56 umol/L after 45 min.	Unchanged from level observed at zero time (16.3 ± 7.4 umol/L) but higher compared to adults even at zero time (1.6 ± 0.5 umol/L)	

Subjects/Study	Doses	Duration	Plasma Phe	Plasma Aspartate	Comments
6 subjects sensitive to Chinese restaurant syndrome/cross over	Loading dose of 34 mg/kg bw aspartame and sucrose (1000 mg/kg bw)		Increased after aspartame loading from baseline (56.6 ± 8.2 umol/L) to a peak of 130 ± 25 umol/L after 30 min. No significant changes after sucrose loading.	No significant differences.	E110, 1979. Effect of aspartame loading in subjects who report symptoms of Chinese restaurant syndrome after glutamate ingestion.
9 healthy subjects (6 males and 3 females)	Soup beverage meal without MSG or aspartame, with MSG (50 mg/kg bw) or with MSG (50 mg/kg bw) plus aspartame (34 mg/kg bw)		Increased significantly after aspartame ingestion; mean peak level of 133 ± 50 umol/L after 30 min of ingestion of the meal, returning to baseline after 4 hours.	Small but significant increases after aspartame ingestion; mean peak values of 49 ± 29 umol/L at 30 min, which rapidly returned to baseline 150 min after aspartame ingestion.	E111, 1979. Metabolic studies of aspartame and monosodium glutamate (MSG) when ingested together as part of a soup beverage meal.
6 healthy subjects (3 males and 3 females)	Hamburger-milk shake alone or with MSG (150 mg/kg bw) or with MSG (150 mg/kg bw) plus aspartame (23 mg/kg bw)		Increased after aspartame ingestion compared to MSG alone; significant peak level of 107 ± 23 umol/L after 3 hours of ingestion of the meal.	Small increases (not significant compared to MSG alone) after aspartame ingestion; peak values of 36 ± 19 umol/L at 1 hour); 32.5 ± 14 umol/L after 1.5 hour ($p \leq 0.05$ compared to MSG alone).	E112, 1979. Metabolic studies of aspartame and monosodium glutamate (MSG) ingested as components of a hamburger-milk shake meal system in normal adult subjects.
6 healthy males	560 mg Phe in the form of 1000 mg aspartame or 12200 mg albumin or water		Increased from 488 ± 96 to 75.8 ± 27.5 umol/L at 0.25 h after aspartame ingestion. AUC: 40% increased (not significantly) after aspartame ingestion: 53 ± 26	Significantly increased at 0.25 h after aspartame ingestion. Peak at 6 umol/L.	Ref: Moller, 1990

Subjects/Study	Doses	Duration	Plasma Phe	Plasma Aspartate	Comments
			umol/L hr (aspartame); 38 ± 15 umol/L hr (albumine)		
8 healthy subjects/ randomised cross- over	500 mg aspartame alone or with 100 g of sucrose		Significantly increased after aspartame ingestion from 62.1 ± 12.0 umol/L (baseline) to 75 ± 13.4 umol/L (15 min after dosing). Not significantly increased from baseline (60.4 ± 11.3 umol/L) after aspartame with sucrose.	Not significantly changed.	Ref: Burns et al., 1991
10 healthy subjects/ randomised cross- over	3000 mg aspartame in solution or in capsules		Significantly increased: peak= 191 ± 65.4 umol/L at 35 ± 15 min, AUC= 15.340 ± 4.820 umol/L/min (solution) vs 117 ± 39.5 umol/L (capsules) at 123 ± 74 min, AUC= 8465 ± 3356 umol/L/min.	Significantly increased: peak= 26.2 ± 16.3 umol/L at 30 ± 14 min (solution) vs 10.4 ± 5 umol/L (capsules) at 106 ± 61.3 min.	Ref: Stegink et al., 1987a
12 healthy subjects	4 mg/kg bw aspartame		Not significantly increased.	Not significantly increased	Ref: Stegink et al., 1987b
20 healthy subjects/ randomised cross- over	20 mg/kg aspartame (capsules or solution)		Significantly increased: C _{max} = 103.3 umol/L at T _{max} = 108.6 min (capsule) vs C _{max} = 126.6 umol/L at T _{max} = 36.6 min (solution). AUCs not significantly different.	Not affected.	Ref: Burns et al., 1990
Healthy subjects	Loading dose of 34, 50, 100, 150, 200 mg aspartame/kg bw		Significantly increased. Peak at 110 ± 25 umol/L, 162 ± 49 umol/L, 203 ± 20.5 umol/L, 351 ± 113 umol/L and 487 ± 151 umol/L after 30 min-2 hours	Not significant differences at 34, 50, 150, 200 mg/kg bw. Statistically significant increase with peak at 4.3 ± 2.3 umol/L at 100 mg/kg bw (after 0.5 h aspartame ingestion). Increased but less than	Ref: Stegink, 1984

Subjects/Study	Doses	Duration	Plasma Phe	Plasma Aspartate	Comments
				those observed post-prandially in infants or adults fed a protein containing meal.	
6 healthy subjects	184 mg aspartame (in a diet coke can), providing 104 mg Phe		Not affected over the 3-hours post ingestion period.		Ref: Mackey and Berlin, 1992
6 healthy women/ cross over	Aspartame or lactose at 50 mg/kg bw		Significantly increased: 4-fold over fasting values 45 min after aspartame loading. Returned to baseline in 4 hours.	Not significant changes.	Ref: Stegink et al., 1979a.
24 one-year old infants	3 4, 50 and 100 mg/kg bw aspartame in a beverage		Significantly increased. Mean peak: 93 ± 14, 116 ± 44, 223 ± 115 umol/L at 34, 50, and 100 mg/kg bw (similar to values observed in adults).	Not significantly changed at 34 and 50 mg/kg bw. Significantly increased at 100 mg/kg bw.	Ref: Filer et al., 1983.
REPEATED DOSES					
31 healthy man and 38 women (21-45 years old)/double blind	Placebo, 200 mg or 300 mg aspartame in capsule. Weekly dose escalation protocol: 0.6, 1.2, 2.4, 4.5, 6.3, 8.1 g aspartame/day.	Six weeks	No significant difference with placebo, fluctuations (mean below 120 umol/L). Peak= 120 umol/L after 9 weeks.	NR	E23, 1972. Short term tolerance of aspartame by normal adults.
95 obese men and women (21-70 years old)/double blind	Placebo, 200 mg or 300 mg aspartame in capsule. Weekly dose escalation protocol: 0.6, 1.2, 2.4, 4.5, 6.3, 8.1 g aspartame/day.	Six weeks	No significant difference with placebo, fluctuations (mean below 120 umol/L). Peak= 120 umol/L after 3 weeks.	NR	E24, 1972. Short term tolerance of aspartame by obese adults.
Group 1: 18 males	1800 mg aspartame/day	27 weeks	No significant difference with	NR	E60, 1973. Long term

Subjects/Study	Doses	Duration	Plasma Phe	Plasma Aspartate	Comments
and 32 females who participate in E23 study. Group 2: 12 males and 5 females.	(2 x 300 mg capsules, 3 x daily).	(group 1); 21 weeks (group 2)	placebo, fluctuations (mean below 120 umol/L). Peak= 110 umol/L (male), 103 umol/L (female), after 1 week.		tolerance of aspartame by normal adults.
62 males and 64 females. Group A: 2-3 year old. Group B: 4-6 year-old. Group C: 7-9 year-old. Group D: 10-12 year-old. Group E:13-20 year-old/double blind	Sucrose or aspartame: 43.7±1.66 (A) 39.5±0.99(B) 58.1±2.83(C) 55±3.57(D) 42.9±1.84 (E) mg asp/kg/day.	13 weeks	No significant difference with sucrose, fluctuations. Peaks (asp): 110 umol/L 1 week after dosing (Group A, B), 114 umol/L 1 week postdosing (Group C), 103 umol/L 3 weeks after dosing (Group D), 96 umol/L 1 week after dosing (Group E).	NR	E61, 1972. Long term tolerance of aspartame by normal children and adolescents.
105 obese adults (21-70 years old). Group 1: 69 subjects who participate in E24 study. Double blind. Group 2: 36 subjects.	1800 mg aspartame/day (2 x 300 mg capsules, 3 x daily).	27 weeks (Group 1); 21 weeks (Group 2)	No significant difference with placebo, fluctuations (mean below 120 umol/L). Peak= 96 umol/L after 8-11 weeks.	NR	E64, 1972. Long term tolerance of aspartame by obese adults.
52 men and women (21-45 years old). Follow up of the E25 study.	1800 mg aspartame/day (2 x 300 mg capsules, 3 x daily).	21 weeks	No significant difference with placebo, fluctuations (mean around 120 umol/L). Peak= 132 umol/L after 1 week. The only significant difference between the treatment groups occurred in the Phe levels at week 16.	NR	E67, 1973. Long term tolerance of aspartame by adults PKU heterozygotes.
6 healthy adult	Test beverage alone or	8 hours/Wash	Significantly increased after	Slightly increased;	UN05, 1987. Effect of repeated

Subjects/Study	Doses	Duration	Plasma Phe	Plasma Aspartate	Comments
subjects/cross over	with aspartame (600 mg/hour or 18 mg/kg bw) or DKP (150 mg/hour or 4.5 mg/kg bw)	out of 1 week between treatments	aspartame ingestion; mean plasma C _{max} = 113 umol/L (aspartame), 58 umol/L (placebo); T _{max} = 5.83 hours (aspartame) and 2.83 hours (placebo); Total AUC (0-9)= 788 umol/L (aspartame), 444 umol/L (placebo). Plateau concentration after 5 servings.	Mean C _{max} = 15 umol/L (aspartame), 10.8 umol/L (placebo). No significant difference for T _{max} and AUC.	ingestion of beverage containing aspartame or DKP on amino acid and DPK concentrations in plasma and urine of normal healthy subjects. Stegink <i>et al</i> , 1989.
53 healthy adult subjects (23 males and 30 females) for aspartame group; 55 healthy subjects (28 males and 27 females) for placebo group/randomise double-blind, placebo-controlled	7 5 mg/kg/day (capsules of 300 mg divided into 3 doses/day)	24 weeks	No statistically significant differences. Mean: 49 ± 18 umol/L after 24 weeks (aspartame); Mean: 45 ± 6 umol/L after 24 weeks (placebo). C _{max} =60 umol/L, T _{max} =9.79 weeks (aspartame); C _{max} =54 umol/L, T _{max} =8.22 weeks(placebo).	No statistically significant changes. Mean: 7.7 ± 5.1 umol/L after 24 weeks (aspartame); Mean: 7.1 ± 2 umol/L after 24 weeks (placebo). C _{max} = 11.1 umol/L T _{max} = 8.85 weeks (aspartame); C _{max} = 10.1 umol/L T _{max} = 9.98 weeks (placebo)	UN 08, 1988. Safety of long term aspartame administration in normal subjects.
8 healthy adults (4 males and 4 females)/randomise d cross-over	10 mg/aspartame bw/serving	3 servings at 2 hours of interval (6 hours)	Significantly increased: 16.4 to 20.5 umol/L above baseline value (51 ± 8.2 umol/L) 30 to 45 minutes after each dose (within normal postprandial value at each time).	Unaffected	Ref: Stegink et al., 1988
6 healthy young adults (3 males and 3 females)/cross-	600 mg aspartame/serving	8 servings at 1 hours of interval (8	Significantly increased: 14.1 to 23.5 umol/L above baseline value, 30 min after ingestion (within normal	Not significantly increased.	Ref: Stegink et al., 1989

Subjects/Study	Doses	Duration	Plasma Phe	Plasma Aspartate	Comments
over		hours)	postprandial value at each time). Steady state after 4-5 serving.		
PKU HETEROZYGOTE					
SINGLE DOSE					
12 healthy subjects/4 female PKU Heterozygotes	Loading dose of 34 mg/kg bw aspartame		Slightly increased from 110 to 160 umol/L after 1 hour but close than those noted postprandially (120 ± 30 umol/L).	Unchanged.	E93, 1977. Effect of aspartame loading upon plasma and erythrocyte free amino acid levels in normal adult subjects.
4 healthy women (20-28 years) and 4 PKU heterozygotes women.	Loading dose of 34 mg/kg bw aspartame		Remained within the normal range (60-182 umol/L) in both healthy and PKU women. Peak at about 140-150 umol/L after 1 hour (PKU heterozygotes) and 50-120 umol/L after 1 hour (normal subjects).	NR	E108, 1978. Effect of aspartame in plasma and red cell amino acids of apparently healthy female adults and on presumed PKU heterozygotes.
6 healthy men and women and 5 PKU heterozygotes women.	Loading dose of 100 mg/kg bw aspartame		Increased after aspartame ingestion in both normal and PKU subjects; mean maximum levels of 200 ± 60 umol/L after 30-90 min (normal subjects); ranging from 355 ± 76 umol/L at 30 min to 417 ± 24 umol/L at 90 min (in PKU subjects).	No significant changes in both normal and PKU subjects.	E109, 1978. Effect of aspartame loading at 100 mg/kg bw upon plasma and erythrocyte levels of free amino acid in normal subjects and subjects presumed to be heterozygotes for PKU.
8 healthy subjects and 6 PKU heterozygotes/randomised cross-over	10 mg/kg bw aspartame		Increased. Healthy subjects: from baseline of 51 ± 8.2 umol/L to 67 ± 7.5 umol/L. PKU: from baseline of 90 ± 17.1 umol/L to 121 ± 20.8 umol/L	Not significantly increased.	Ref: Stegink et al., 1987b

Subjects/Study	Doses	Duration	Plasma Phe	Plasma Aspartate	Comments
12 healthy subjects (6 men and 6 women) and 8 PKU heterozygotes women	Loading dose of 34 mg aspartame/kg bw		Significantly increased. Peak at 110 umol/L after 1 hour (normal) and 160 umol/L after 1 hour (PKU).	Not affected.	Ref: Filer and Stegink 1989
13 heterozygotes (7 women and 6 men) and 13 healthy subjects (5 women and 8 men)	PKU (7 women and 6 men) and 13 healthy subjects (5 women and 8 men) A meal providing 303 umol/kg Phe		Increased. Mean peak around 100 ± 10 umol/L after 3 hours (PKU); around 80 ± 10 umol/L after 3 hours (control).	NR	Ref: Curtius et al., 1994
10 heterozygotes (5 women and 5 men) and 10 healthy subjects (5 women and 5 men)	PKU (5 women and 5 men) and 10 healthy subjects (5 women and 5 men) Same meal with addition of 85 umol/kg aspartame (providing 75 umol/kg Phe)		Increased. Mean plasma level: 95 ± 7 umol/L after 1 hour (control); 153 ± 21 umol/L after 3 hours (PKU heterozygous). Only slightly greater than the usual postprandial range for both controls and PKU heterozygotes.	NR	
10 healthy subjects as control; subjects with classic PKU; subjects with atypical hyperphenylalaninemia; subjects PKU heterozygotes	10 mg/kg bw aspartame		Significantly increased from 44.5 ± 12.9 umol/L (baseline) to 58.0 ± 9.5 umol/L (1 hour after ingestion) in controls (+ 30%); from 68.8 ± 13.8 umol/L (baseline) to 82.3 ± 17.3 umol/L (1 hour after ingestion) in PKU heterozygotes (+ 20%)	NR	Ref: Caballero et al., 1986
6 heterozygotes females and 12	PKU 34 mg/kg aspartame in orange juice		Increased. Healthy subjects: from 56.6 ± 12.1 umol/L (baseline) to 111 ± 25 umol/L (within normal	Not affected	Ref: Stegink et al., 1979b.

Subjects/Study	Doses	Duration	Plasma Phe	Plasma Aspartate	Comments
healthy subjects (6 males and 6 females)			postprandial range); returned to baseline after 8 hours. PKU subjects: mean peak at 160 ± 22.5 umol/L (only slightly above postprandial values in human infant and adult).		
REPEATED DOSES					
65 men and women PKU heterozygotes (21-45 years old)/double blind	Placebo, 200 mg or 300 mg aspartame in capsule. Weekly dose escalation protocol: 0.6, 1.2, 2.4, 4.5, 6.3, 8.1 g aspartame/day; 0.34, 0.67, 1.35, 2.53, 3.54, 4.55 g Phe/day.	6 weeks	No significant difference with placebo, fluctuations (mean below 120 umol/L). Peak= 168 umol/L after 6 weeks.	NR	E25, 1972. Short term tolerance of aspartame by adults PKU heterozygotes.
6 heterozygotes PKU adult subjects/cross over	Test beverage alone or with aspartame (600 mg/hour or 18 mg/kg bw) or DKP (150 mg/hour or 4.5 mg/kg bw)	8 hours/Wash out of 1 week between treatments	Significantly increased after aspartame ingestion; mean plasma C_{max} = 167 umol/L (aspartame), 75.2 umol/L (placebo); T_{max} = 6.83 hours (aspartame) and 3.08 hours (placebo); Total AUC (0-9)= 1162.6 umol/L (aspartame), 599 umol/L (placebo). Plateau concentration after 6 servings. Small increased after DKP ingestion (not clinically significant changes).	Unaffected	UN06, 1987. Effect of repeated ingestion of beverage containing aspartame or DKP on amino acid and DPK concentrations in subjects heterozygotes for PKU.
6 adults PKU heterozygous/rando	600 mg aspartame/serving	8 servings at 1 hour of	Significantly increased from 23.5 to 40.3 umol/L above baseline 30 min	Not affected	Ref: Stegink et al., 1990

Subjects/Study	Doses	Duration	Plasma Phe	Plasma Aspartate	Comments
mised cross-over		interval (8 hours)	after ingestion. Steady state after 5 serving (slightly but significantly higher than usual postprandial values for heterozygotes PKU).		
48 adults (21 men and 27 women) PKU heterozygotes/ randomised, double blind, placebo-controlled, crossover	Placebo or 15 or 45 mg/kg day	12 weeks	Significantly increased at 1 and 3 hours following ingestion of 45 mg/kg day aspartame. Placebo: 90.4 ± 21.23 umol/L (1 hour post dose) and 88.7 ± 22.36 umol/L (3 hours post dose). 45 mg/kg day group: 116.9 ± 29.48 umol/L (1 hour post dose) and 112.5 ± 40.30 umol/L (3 hours post dose)	NR	Ref: Trefz et al., 1993
PKU HOMOZYGOTE					
SINGLE DOSE					
2 14 year-old PKU homozygous boys: one on a liberalised Phe diet (70 mg/kg/day), the other on a restricted Phe diet (17 mg/kg/day).	Loading dose of 34 mg/kg bw aspartame; 2 weeks later: equivalent amount of Phe (19 mg Phe/kg). Liberalised diet: total amount of 3228 mg of Phe (dietary intake: 2539 mg + Phe added from loading dose: 689 mg); restricted diet: total amount of 2037 mg of Phe (dietary intake: 965 mg + Phe added from loading dose: 1072).		No significant differences reported.	NR	E26, 1972. Tolerance of loading doses of aspartame by PKU Homozygous children.
7 subjects	200 mg aspartame in		Not increased over baseline values	NR	Ref: Wolf-Novak et al., 1990

Subjects/Study	Doses	Duration	Plasma Phe	Plasma Aspartate	Comments
homozygous for PKU and 7 healthy subjects	beverage with or without carbohydrate (CHO)		(55 ± 8.5 umol/L, healthy; 1500 ± 230 umol/L, PKU).		
5 homozygous for PKU subjects	184 mg aspartame (in a diet coke can), providing 104 mg Phe		Increased levels vary from 15.6 umol/L (above BL = 302 umol/L) to 106 umol/L (above BL= 798 umol/L)		Not considered clinically significant. Ref: Mackey and Berlin, 1992

6671 NR= not reported
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6674 **K. SAS INTERNAL TECHNICAL REPORT**6675 **ESTABLISHING LOWER BOUND ASPARTAME DOSE TO REACH PHENYLALANINE**
6676 **PLASMA THRESHOLD LEVELS**6677 **European Food Safety Authority²⁷**

6678 European Food Safety Authority (EFSA), Parma, Italy

6679 **EXECUTIVE SUMMARY**

6680 Simulation models are a powerful tool when dealing with summary data, it can combined several sources of
6681 variation that could arise from the outcome of interest in itself, due to diversity in the population under study, but
6682 also on the limited or scarce amount of information from which the summary measures are built. Moreover when
6683 the purpose of the study is to establish the kind of relationship between outcomes and the covariates of interest,
6684 this type of models also offers the advantage of generating (mimicking) the experiment conditions many times,
6685 and thus accounting for possible variations that could occur if the experiment was conducted several times.
6686 Summary data collected on C-max phenylalanine plasma concentration for different studies on different doses
6687 were used to estimate the minimum (lower bound) dose of aspartame that is obtained for a particular
6688 phenylalanine plasma level threshold. The data contain arithmetic means and standard deviation of
6689 phenylalanine levels in plasma (μM), number of individuals involved and the aspartame dose administered
6690 (mg/kg of body weight). The arithmetic measures were transformed to obtained geometric measures. In each
6691 simulation individual phenylalanine levels in plasma for each dose were generated according to the number of
6692 individuals reported, geometric mean and the geometric standard deviations based on a lognormal. For each
6693 simulated dataset a generalized additive model (Wood 2003, 2004, 2006a, 2006b and 2011), considering the
6694 smoothing functions to be spline functions of aspartame doses was used. The simulation model used allows
6695 studying the relationship between aspartame dose (mg/kg) and phenylalanine plasma level (μM) for healthy and
6696 heterozygote individuals. Flexible models were used to fit the simulated individual data based on geometric
6697 transformations of the summary measures drawn from lognormal distribution, the smoothed fitted model was
6698 evaluated in term of monotonicity and results shown that the model obtained was strictly increasing monotone,
6699 even when the model structure was left flexible, allowing for other type of relationship, but the data at hand
6700 proved to be monotonically increasing. The results obtained for the three different threshold used (240, 300 and
6701 360 in μM) in normal individuals consistently produced aspartame dose lower bound that were above the ADI
6702 level (40 mg/kg of body weight) regardless of the confidence level (α) used. In the case in which the model was
6703 fitted for heterozygote individuals, thresholds of 300 or 360 μM shown consistent aspartame dose lower bounds
6704 above the ADI level when the level of confidence considered was 0.05, and only for the threshold of 360 when α
6705 was equal to 0.01. If the threshold considered is 240 μM and α is 0.05, the aspartame dose lower bound could be
6706 below the ADI level (in about 18 % of the times), for the case in which the confidence level considered was
6707 0.01, then in 46.6% of the simulations, the aspartame dose lower bound was below the ADI level for the
6708 phenylalanine threshold of 240 μM and 5.1% for the phenylalanine threshold of 300 μM . It is important to
6709 highlight that for the heterozygote population, the results obtained should be interpreted with caution due to the
6710 limited number of doses found and the amount of individuals used to derive the summary data.

6711

6712 **KEY WORDS**

6713 Spline model, dose-response models,

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27 Correspondence: jose.CORTINASABRAHANTES@efsa.europa.eu

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6716 **BACKGROUND**

6717 The Commission has asked the European Food Safety Authority to re-evaluate the safety of food additives
6718 already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking
6719 especially into account the priorities, procedure and deadlines that are enshrined in the Regulation (EU) No
6720 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in
6721 accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food
6722 additives.

6723 **OBJECTIVES AND SCOPE OF THE REPORT**

6724 The objective of this report is to:

- 6725 • Provide assistance to establish the dose lower bounds for different phenylalanine plasma level
6726 thresholds
- 6727 • Develop a simulation model that uses summary information regarding C-max phenylalanine
6728 plasma level for certain aspartame bolus doses collected from different studies in humans that
6729 account for the variability in each dose groups and the limited number of individuals used to
6730 estimate the phenylalanine C-max levels to estimate the dose lower bounds for the pre-
6731 specified thresholds.
6732

6733 **1. Introduction**

6734 Simulation models are a powerful tool when dealing with summary data, it can combined several
 6735 sources of variation that could arise from the outcome of interest in itself, due to diversity in the
 6736 population under study, but also on the limited or scarce amount of information from which the
 6737 summary measures are built. Moreover when the purpose of the study is to establish the kind of
 6738 relationship between outcomes and covariates of interest, these type of models also offers the
 6739 advantage of generating (mimicking) the experiment conditions more than once, and thus accounting
 6740 for possible variations that could occur if the experiment was conducted several times. Spline models
 6741 (Wood 2003, 2004, 2006a, 2006b and 2011) is a non/semi-parametric flexible tool that could be used
 6742 in order to establish the relationship between the outcome variable under consideration and the
 6743 covariates that could potentially have an effect on the outcome observed, offering the possibility to
 6744 obtained smoothing curves without having to specify any predefined mathematical expression. The
 6745 SAS unit was asked to conceive a model that could allow the estimation of the aspartame dose lower
 6746 bounds for different phenylalanine plasma level threshold that could arise when modelling the
 6747 relationship between aspartame bolus doses and C-max phenylalanine level in plasma. For this
 6748 purpose simulation models in combination with spline modelling techniques are proposed.

6749 The objective of this report is to:

6750 • Provide assistance to establish the dose lower bounds for different phenylalanine
 6751 plasma level threshold

6752 • Develop a simulation model that uses summary information regarding C-max
 6753 phenylalanine plasma level for certain aspartame bolus doses collected from different
 6754 studies in humans that account for the variability in each dose groups and the limited
 6755 number of individuals used to estimate the phenylalanine C-max levels to estimate the
 6756 dose lower bounds for the pre-specified thresholds.

6757 **2. Methodology Proposed**

6758 Summary data collected on C-max plasma concentration for different studies on different doses were
 6759 used to estimate the minimum (lower bound) dose of aspartame that is obtained for a particular
 6760 phenylalanine plasma level threshold. The data contain arithmetic means (\bar{y}) and standard deviation
 6761 (S_y) of phenylalanine levels in plasma (y in μM), number of individuals involved (n) and the
 6762 aspartame dose administered (d in mg/kg of body weight) . The data were taken from the following
 6763 sources.

6764 Burns ST, Stargel, WW and Horwitz A, 1990. Bioavailability of phenylalanine and aspartate from
 6765 aspartame (20 mg/kg) in capsules and solution. *Metabolism*, 39, 1200-1203.

6766 Stegink LD, Wolf-Novak LC, Filer LJ Jr, Bell EF, Ziegler EE, Krause WL and Brummel MC, 1987.
 6767 Aspartame-sweetened beverage: effect on plasma amino acid concentrations in normal adults and
 6768 adults heterozygous for phenylketonuria. *The Journal of Nutrition*, 117, 1989-1995.

6769 E93, Final Report On Aspartame Studies - Effect Of Aspartame Loading Upon Plasma And
 6770 Erythrocyte Free Amino Acid Levels In Normal Adult Subjects. Searle, 1977.

6771 E108, Effect of aspartame on plasma and red cell amino acids of apparently healthy female adults and
 6772 on presumed phenylketonuric heterozygotes. Searle, 1978.

6773 E110, Effect of Aspartame Loading In Subjects Who Report Symptoms Of Chinese Restaurant
 6774 Syndrome After Glutamate Ingestion. Searle, 1979.

6775 The arithmetic measures were transformed to obtained geometric measures (\bar{z} and S_z), since it is
 6776 believed that plasma concentration of phenylalanine levels should follows a lognormal distribution
 6777 ($z = \ln(y)$). The transformation was done as follows:

$$CV_y = \frac{S_y}{\bar{y}}$$

$$\bar{z} = \ln \left[\frac{\bar{y}}{\sqrt{1 + CV_y^2}} \right]$$

$$S_z^2 = \ln(1 + CV_y^2)$$

6778 Simulation models (1000 simulations) were used in order to estimate the dose lower bound for three
 6779 different phenylalanine plasma level thresholds (240, 300 and 360 μM) and different significance (α)
 6780 levels (0.05 and 0.01).

6781 In each simulation individual phenylalanine levels in plasma for each dose were generated according
 6782 to the number of individuals reported, geometric mean (\bar{z}) and the geometric standard deviations (S_z)
 6783 based on a lognormal distribution with these parameters (\bar{z} and S_z). For each simulated dataset a
 6784 generalized additive model (Wood 2003, 2004, 2006a, 2006b and 2011), considering the smoothing
 6785 functions to be spline functions of aspartame doses was fitted and the confidence band around the
 6786 fitted model was used to estimated the α -percentile lower bound for aspartame dose that can
 6787 potentially achieved the three different phenylalanine plasma level thresholds (240, 300 and 360 μM).

6788 A generalized additive model (GAM, Hastie and Tibshirani, 1986, 1990) is a generalized linear model
 6789 (GLM, McCullagh and Nelder, 1989) where the linear predictor is specified as a sum of smooth
 6790 functions of some or all of the covariates collected.

$$E(Z_i) \equiv \mu_i, \quad g(\mu_i) = \eta_i \equiv D_i^* \beta^* + f_1(d_{1i}) + f_2(d_{2i}) + \dots$$

6791 where Z_i is the response variable; $g(\cdot)$ is a monotonic link function; D_i^* is the i th row of the strictly
 6792 parametric part of the model (called design matrix) and β^* the parameter vector expressing the
 6793 parametric association between the response and the covariate of interest, and the $f_j(\cdot)$ are the smooth
 6794 functions of the covariates d_j , in this particular report thin plate regression spline is used. In order to
 6795 avoid over fitting the model is estimated using penalized maximum likelihood approach. Ample
 6796 details on the method used to fit the model in this report are presented in Wood (2004).

6797 The process was repeated 1000 times and the α -percentile lower bound for aspartame dose were
 6798 retained to finally construct the distribution of lower bounds for each of the three different
 6799 phenylalanine plasma level thresholds, which account for the uncertainty on the observed summary
 6800 levels reported in terms of limited number of subjects for each of the aspartame dose groups.

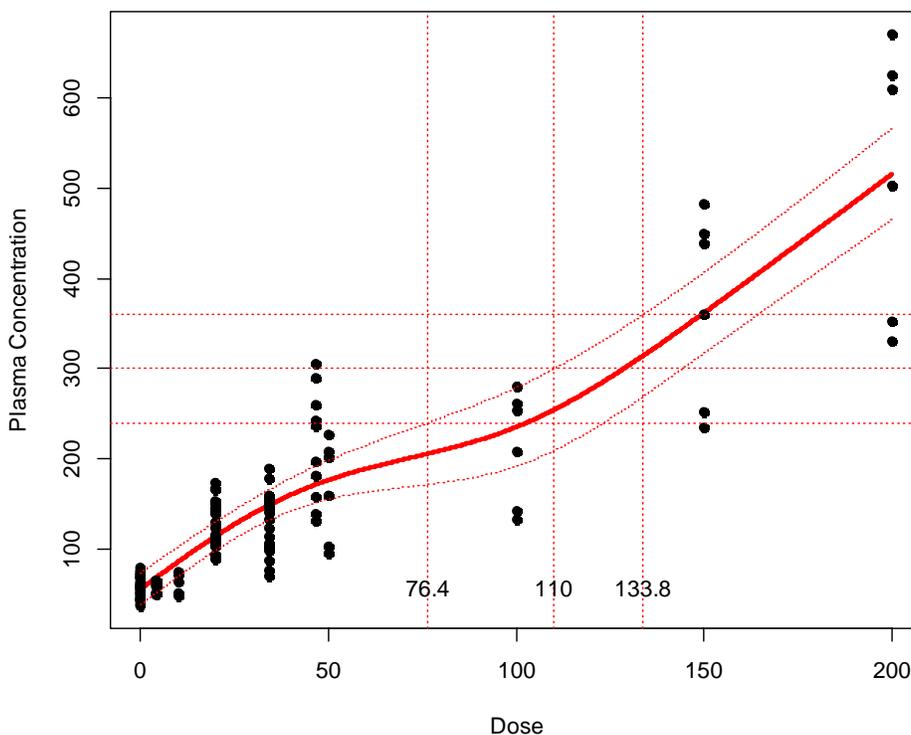
6801 3. Results

6802 3.1. Normal Population

6803 The simulation model in place generate individual C-max phenylalanine plasma level for each of the
 6804 dose groups collated from the different studies based on a lognormal distribution, then the spline
 6805 model is fitted to the individual phenylalanine plasma level data considering thin plate smoothing
 6806 spline function of dose, with smoothing parameter based on cross-validation (**Figure 1** and **Figure 6**,
 6807 considering α to be equal to 0.05 and 0.01 respectively). It is important to highlight the advantage of
 6808 this model is that the type of relationship between the response (C-max phenylalanine plasma level)

6809 and the covariate (aspartame dose) is left unspecified, meaning that not deterministic/mathematical
 6810 models are assumed. The confidence band around the smoothed fitted line is estimated and the
 6811 aspartame doses for which the phenylalanine plasma level thresholds crosses the upper bound of the
 6812 fitted smoothed line is obtained, for example in **Figure 1** when the threshold considered is 240 μM ,
 6813 the associated aspartame lower dose would be 76.4 mg/kg of body weight. In order to explore
 6814 monotonicity of the fitted smooth function derivatives of the function were estimated for each
 6815 simulation, which were strictly greater than zero for all doses between 0 and 200, results for a single
 6816 simulation are shown in **Figure 2**.

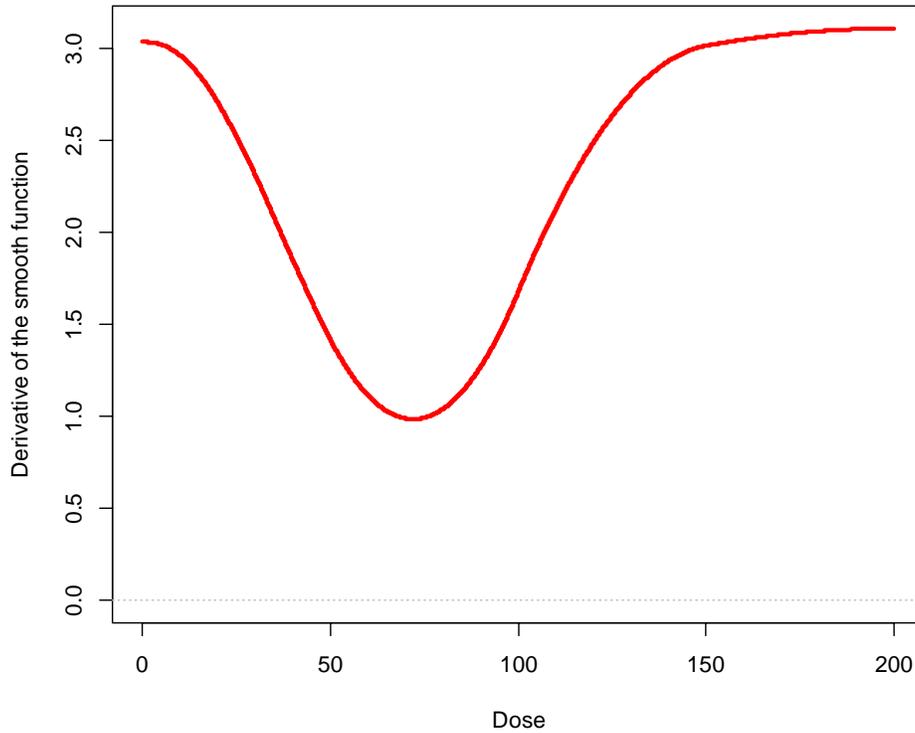
6817 **Figure 3 to Figure 5 and Figure 7 to Figure 9** shown the aspartame dose lower bound distribution
 6818 when the phenylalanine plasma threshold levels are 240, 300 and 360 considering different level of
 6819 confidence (α). It is important to highlight that when the confidence band around the smoothed line is
 6820 using a confidence level of 0.95 ($1 - \alpha$), then for all threshold levels of phenylalanine the distribution
 6821 of aspartame dose lower bound is always above the ADI value of 40 mg/kg. It should also be noted
 6822 that even when $\alpha = 0.01$ and the phenylalanine threshold is considered to be 240 μM , the aspartame
 6823 dose lower bound 95th confidence interval (54.6; 118.1) is above the ADI value of 40 mg/kg.



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6825 **Figure 1.** Single simulation example considering α being equal to 0.05.

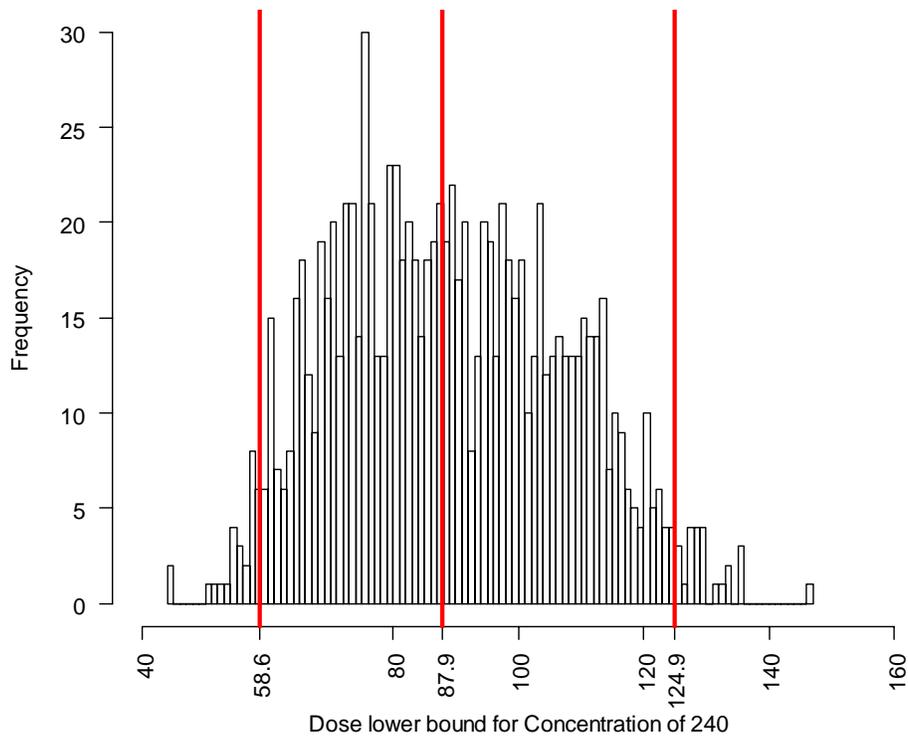
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Figure 2. Derivative of the smoothed function for a single simulation example considering α being equal to 0.05.

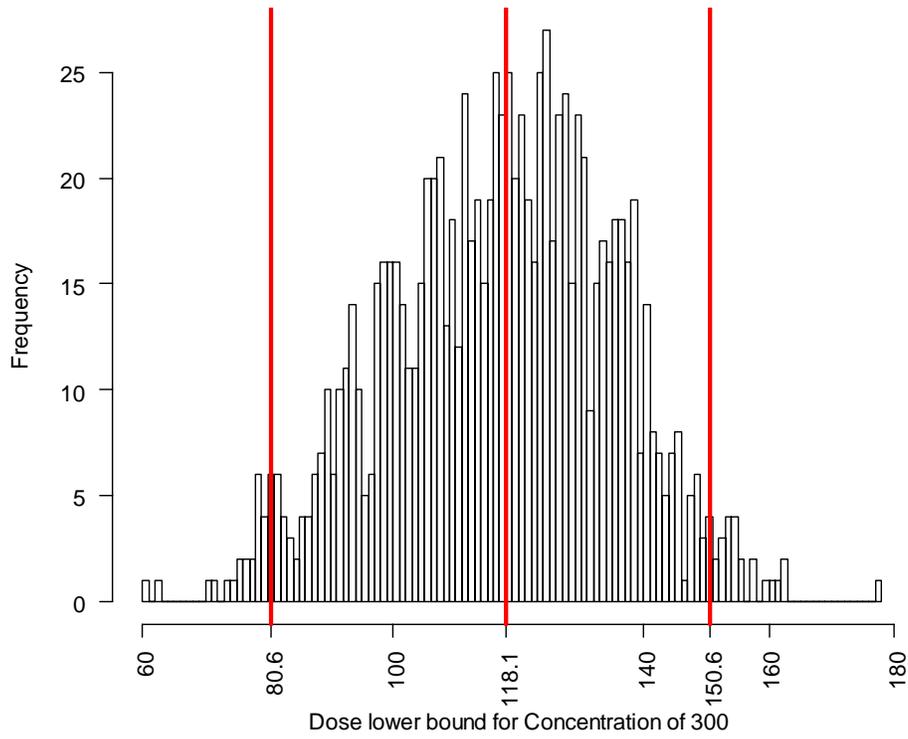
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Figure 3. Histogram of aspartame dose lower bound (considering $\alpha = 0.05$) for phenylalanine plasma level threshold of 240 μM .

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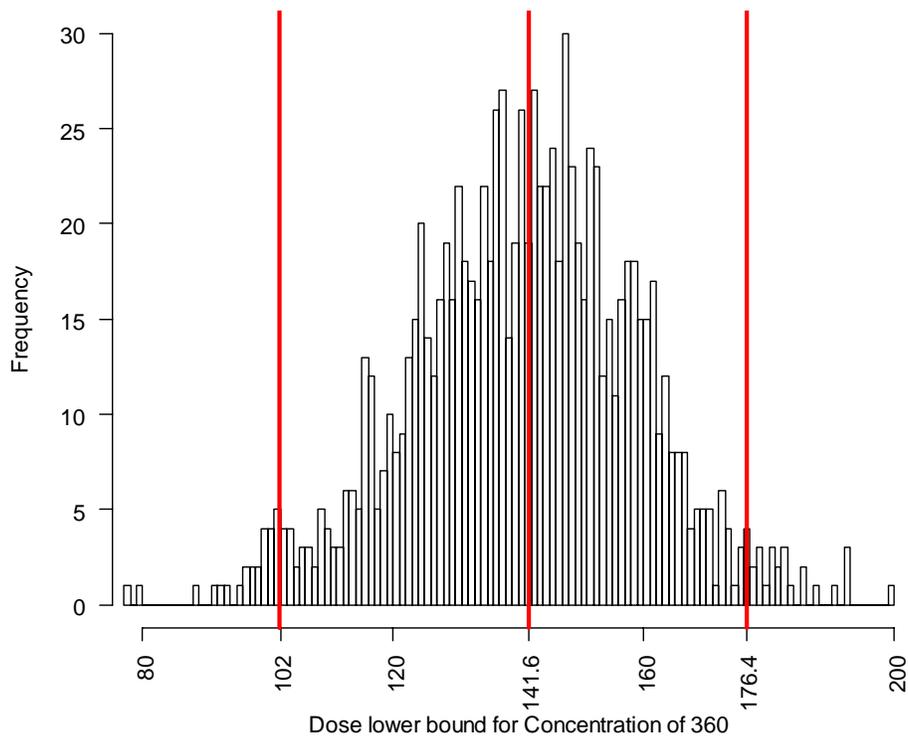
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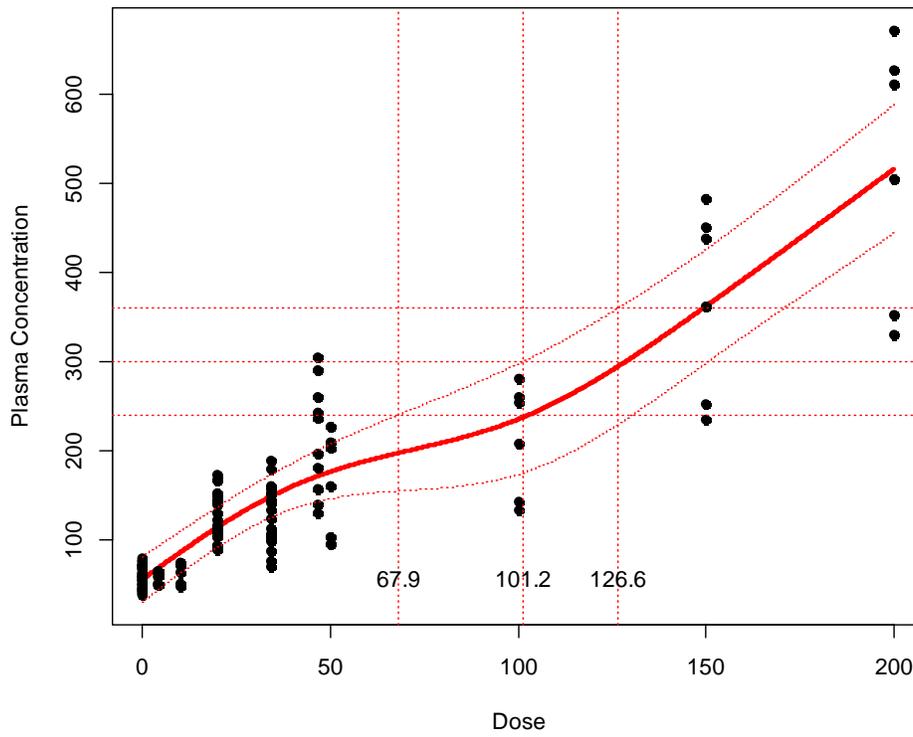
Figure 4. Histogram of aspartame dose lower bound (considering $\alpha = 0.05$) for phenylalanine plasma level threshold of 300 μM .

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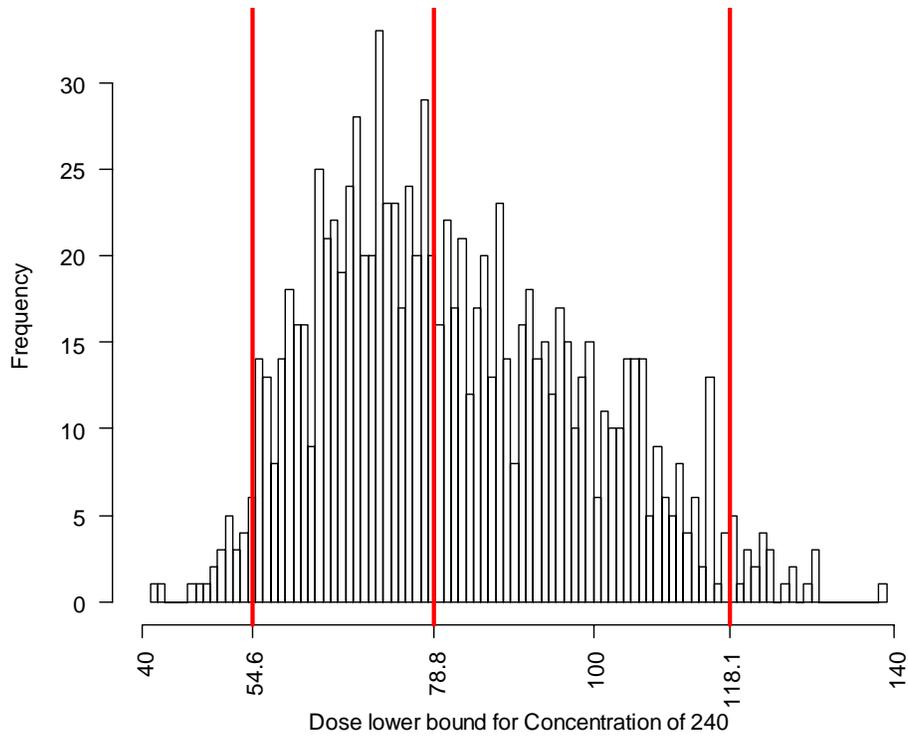
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6840 **Figure 5.** Histogram of aspartame dose lower bound (considering $\alpha = 0.05$) for phenylalanine plasma
6841 level threshold of $360 \mu\text{M}$.



6842
6843 **Figure 6.** Single simulation example considering α being equal to 0.01.

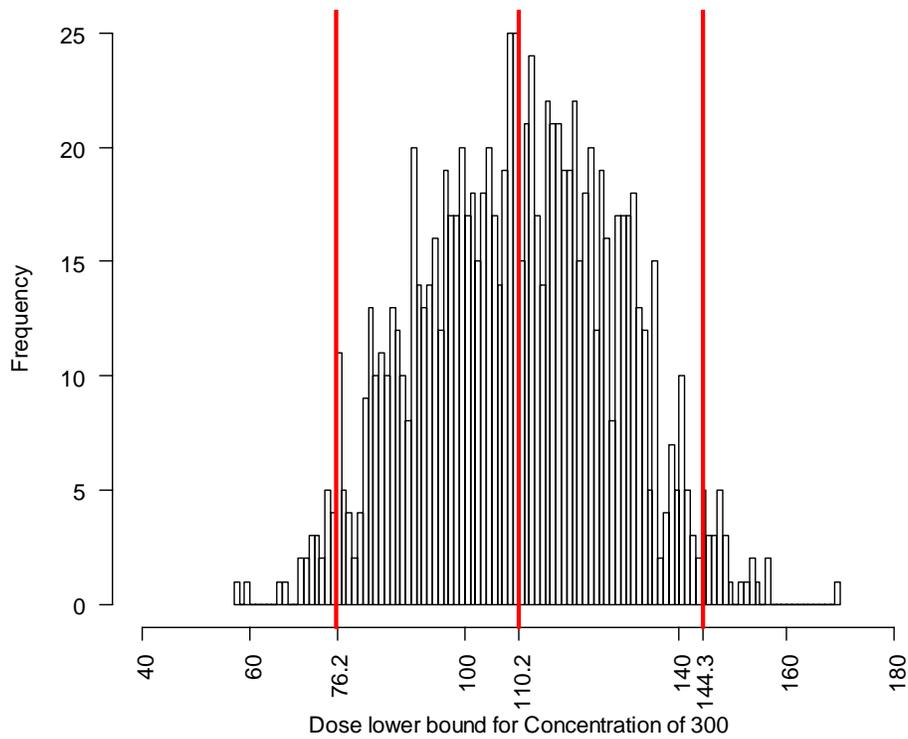
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Figure 7. Histogram of aspartame dose lower bound (considering $\alpha = 0.01$) for phenylalanine plasma level threshold of 240 μM .

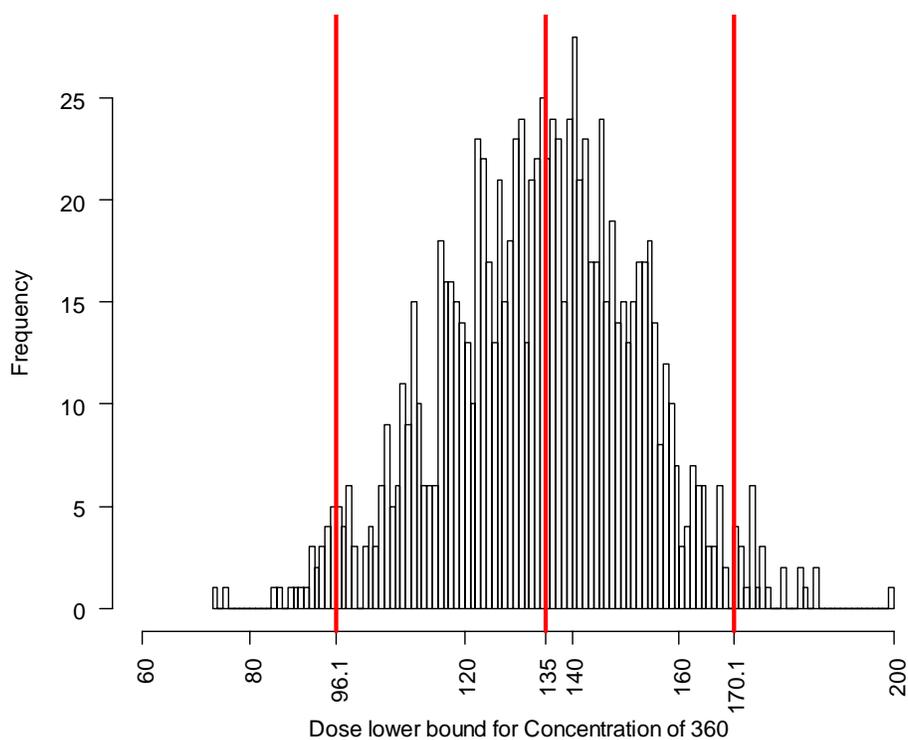
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Figure 8. Histogram of aspartame dose lower bound (considering $\alpha = 0.01$) for phenylalanine plasma level threshold of 300 μM .

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Figure 9. Histogram of aspartame dose lower bound (considering $\alpha = 0.01$) for phenylalanine plasma level threshold of 360 μM .

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3.2. Heterozygote Population

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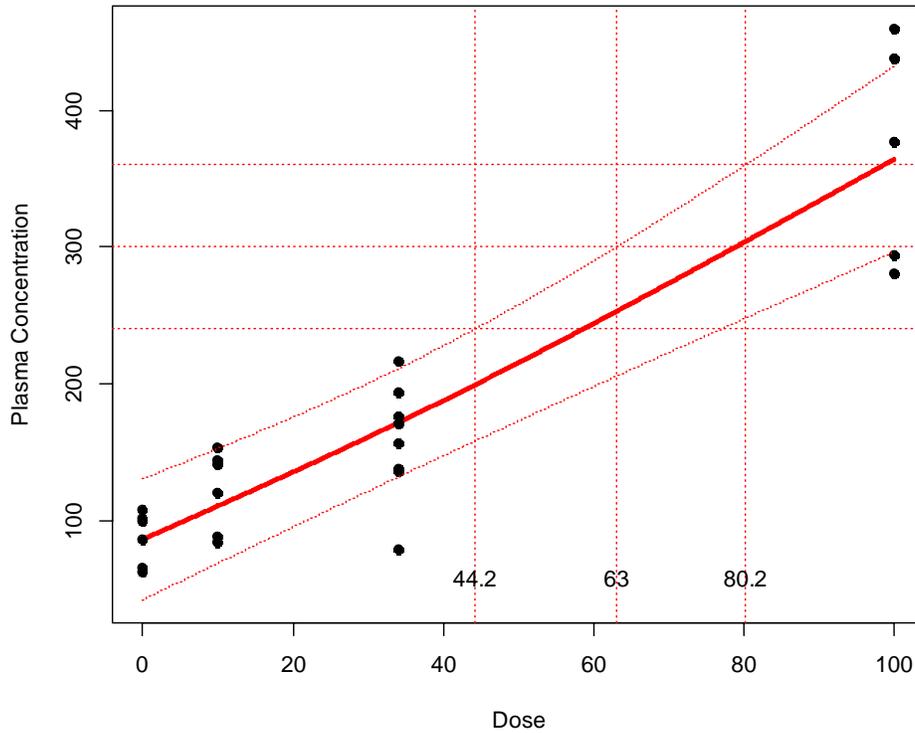
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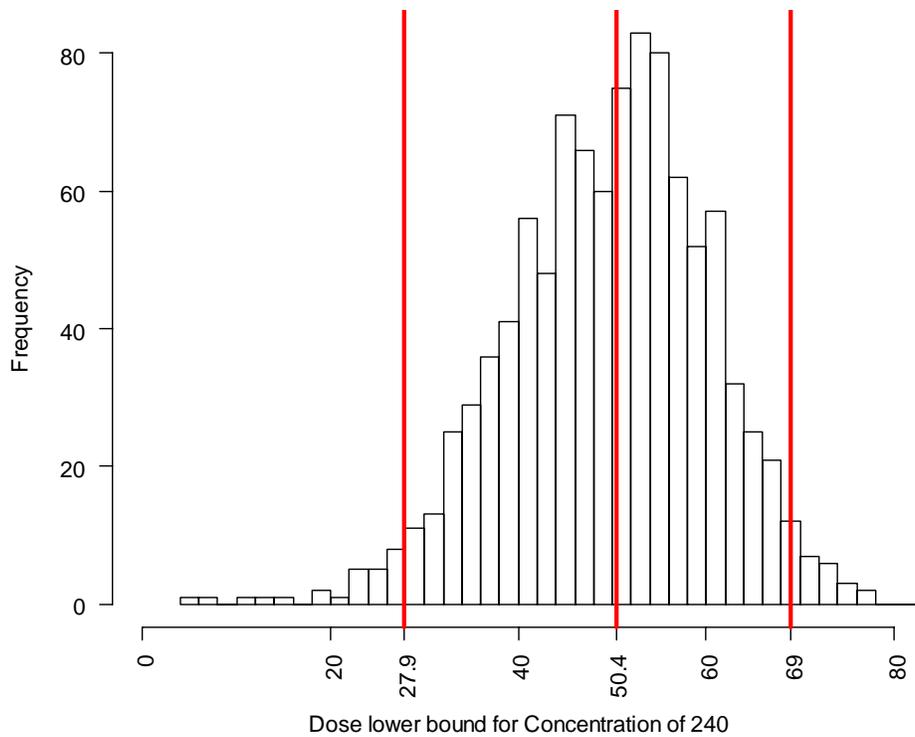
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Similarly the model was fitted for the heterozygote population; it should be highlighted that only information on doses 0, 10, 34 and 100 was obtained for this group, thus results should be interpreted with caution. The results obtained when the level of confidence (α) was 0.05 are shown in **Figure 10** to **Figure 13**. It can be seen that aspartame dose lower bound for the phenylalanine threshold of 240 μM could be between 27.9 and 69, having a lower bound confidence interval below the ADI level. It is important to note that around 18.1% of the simulated data resulted in an aspartame dose lower bound below the ADI level. For the other thresholds (300 μM and 360 μM) the confidence interval obtained did not contain the ADI value, representing only 0.8 % and 0% respectively of the total simulations run. When the simulation was repeated considering the level of confidence to be 0.01 (**Figure 14** to **Figure 17**), then in 46.6% of the simulations, the aspartame dose lower bound was below the ADI level for the phenylalanine threshold of 240 μM , 5.1% for the phenylalanine threshold of 300 μM and 0.5% for the phenylalanine threshold of 360 μM .



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6870 **Figure 10.** Single simulation example considering α being equal to 0.05.



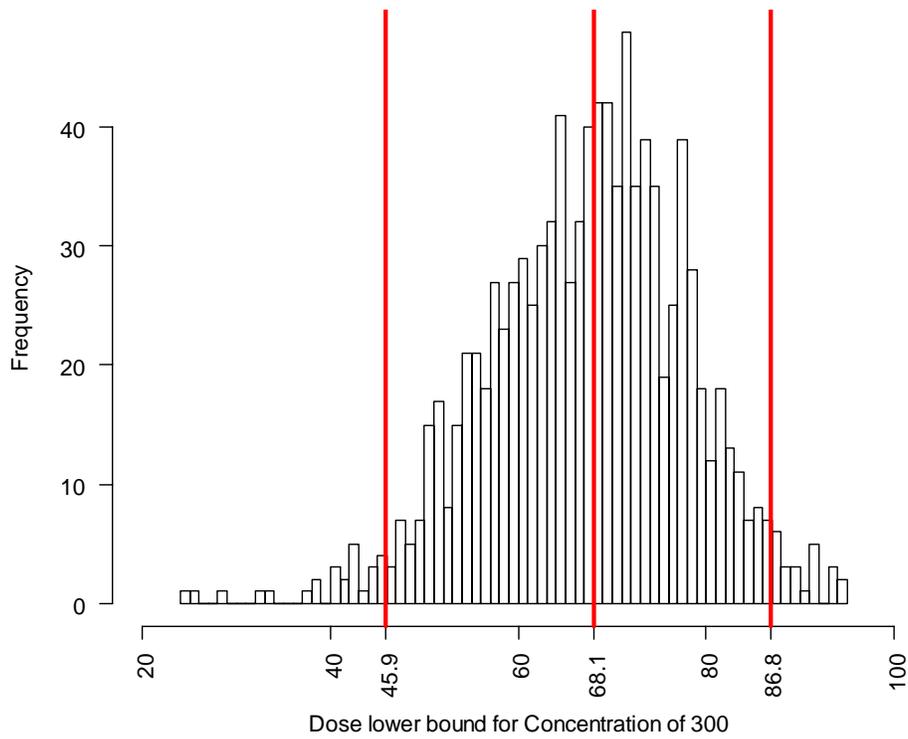
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Figure 11. Histogram of aspartame dose lower bound (considering $\alpha = 0.05$) for phenylalanine plasma level threshold of 240 μM .

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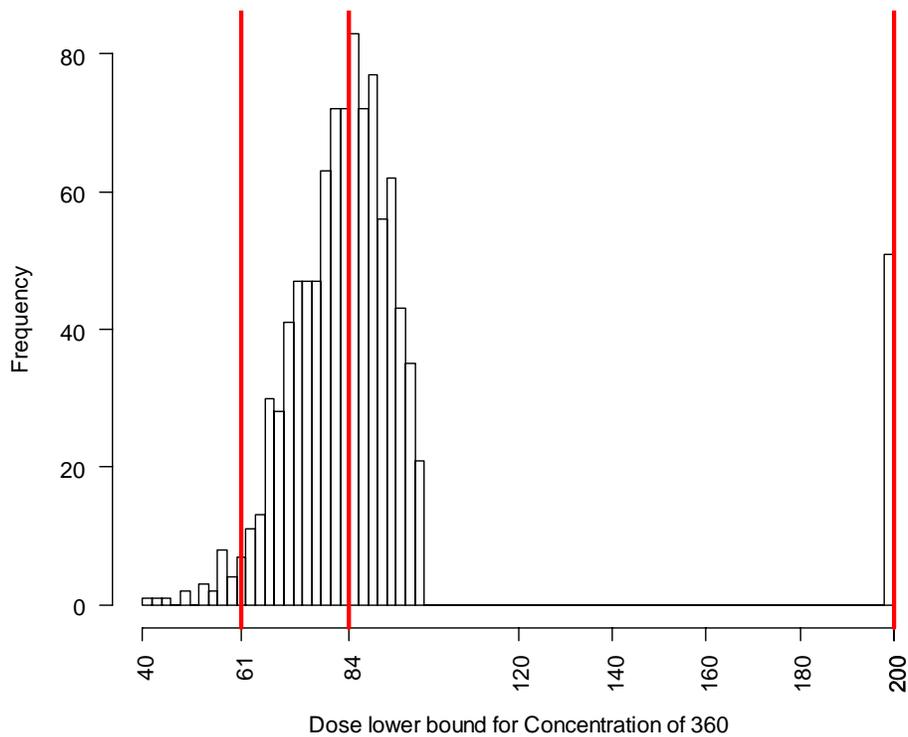
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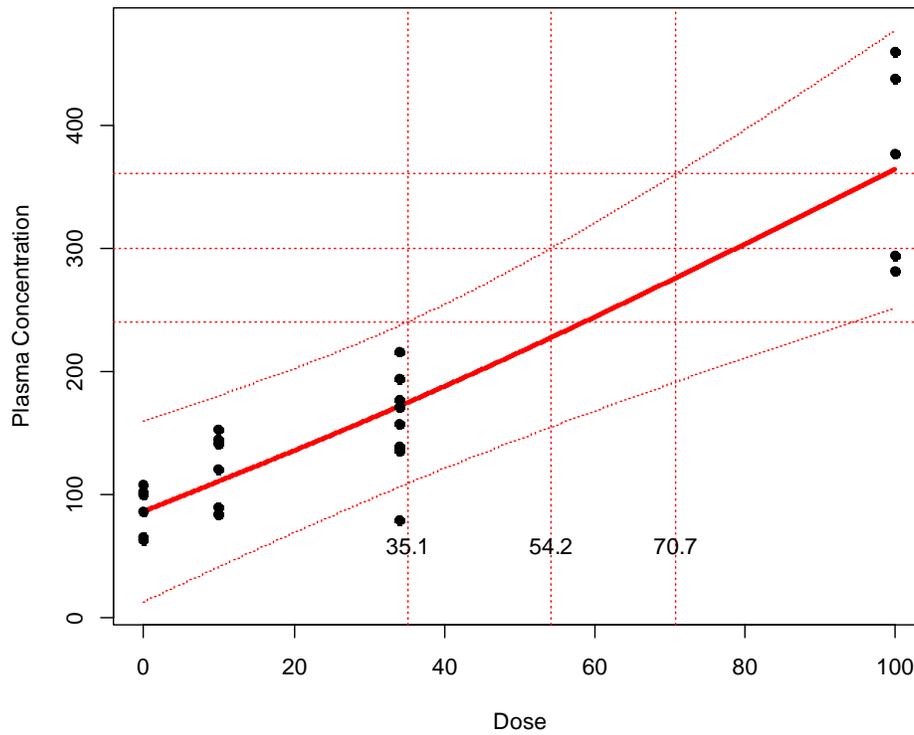
Figure 12. Histogram of aspartame dose lower bound (considering $\alpha = 0.05$) for phenylalanine plasma level threshold of 300 μM .

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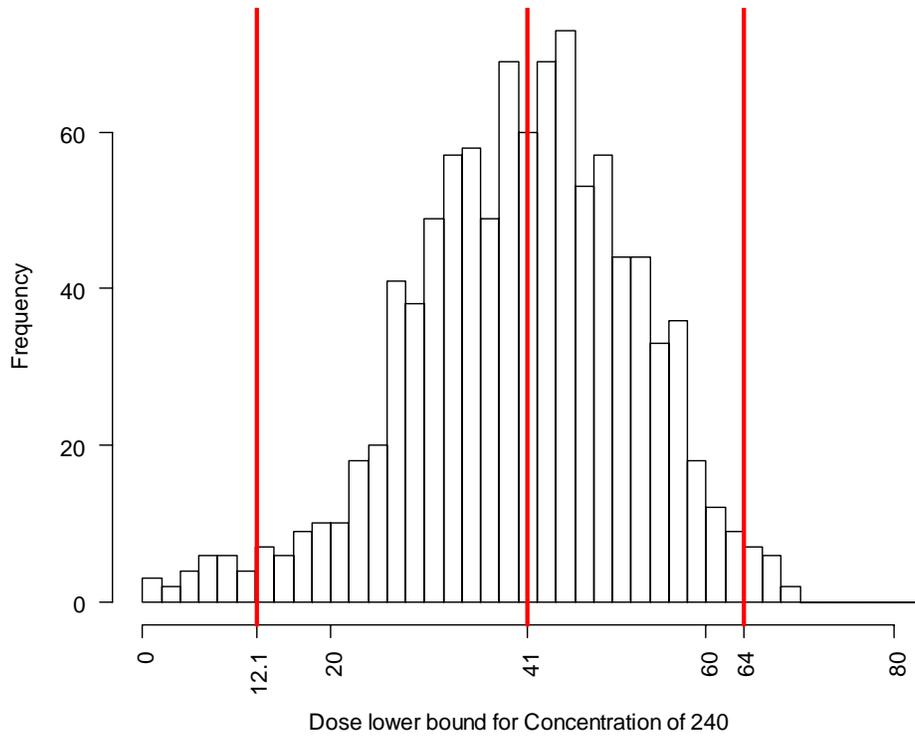
6880 **Figure 13.** Histogram of aspartame dose lower bound (considering $\alpha = 0.05$) for phenylalanine
6881 plasma level threshold of $360 \mu\text{M}$.



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6883 **Figure 14.** Single simulation example considering α being equal to 0.01.

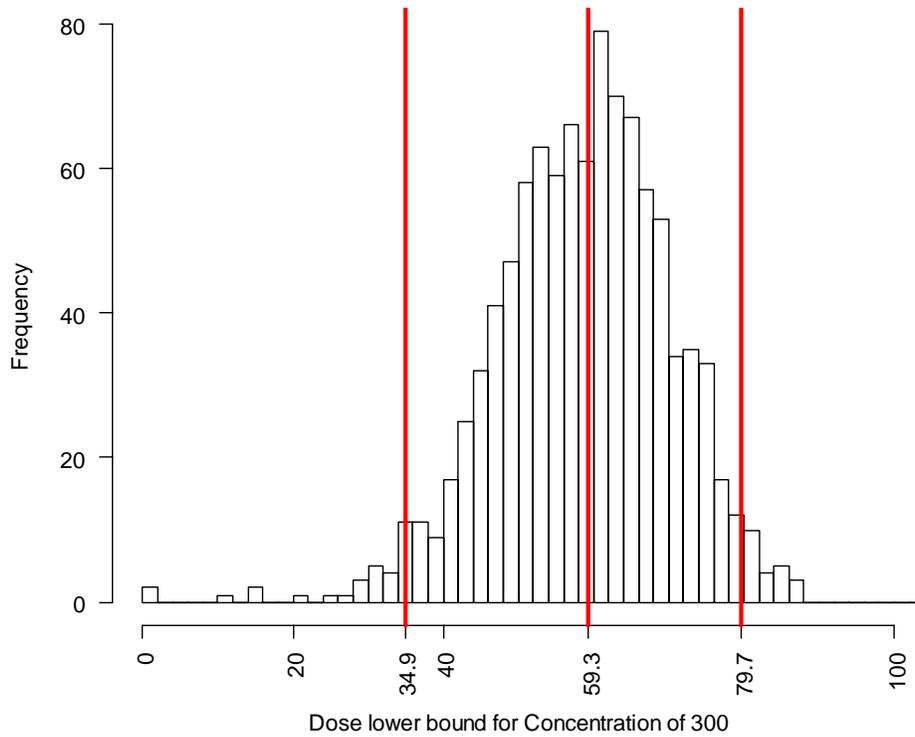
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Figure 15. Histogram of aspartame dose lower bound (considering $\alpha = 0.01$) for phenylalanine plasma level threshold of 240 μM .

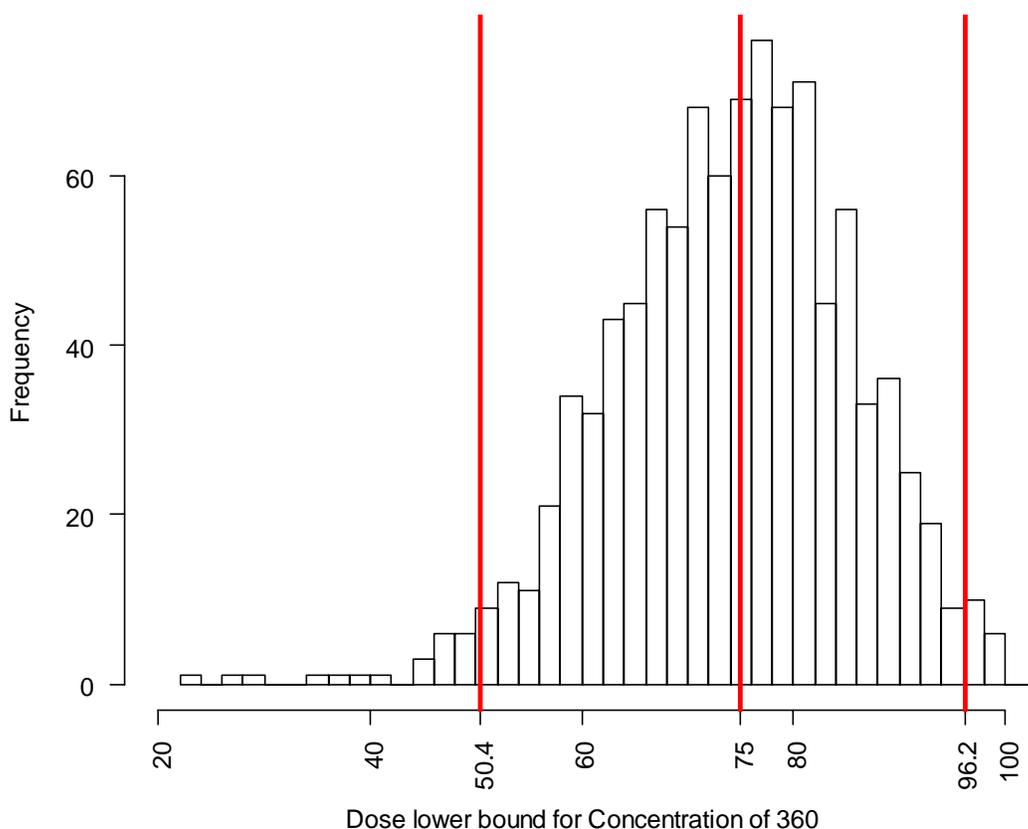
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Figure 16. Histogram of aspartame dose lower bound (considering $\alpha = 0.01$) for phenylalanine plasma level threshold of 300 μM .

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Figure 17. Histogram of aspartame dose lower bound (considering $\alpha = 0.01$) for phenylalanine plasma level threshold of 360 μM .

6896 **CONCLUSIONS**

6897 The simulation model used allows studying the relationship between aspartame dose (mg/kg) and
6898 phenylalanine plasma level (μM). Flexible models were used to fit the simulated individual data based
6899 on geometric transformations of the summary measures drawn from lognormal distribution. The
6900 smoothed fitted model was evaluated in term of monotonicity and results shown that the model
6901 obtained was strictly increasing monotone, even when the model was left flexible, allowing for other
6902 type of relationship, but the data at hand proved to be monotonically increasing. The results obtained
6903 for the three different threshold used (240, 300 and 360 in μM) consistently produced aspartame dose
6904 lower bound that were above the ADI level (40 mg/kg of body weight) regardless of the confidence
6905 level (α) used. In the case in which the model was fitted for heterozygote individuals, thresholds of
6906 300 or 360 μM shown consistent aspartame dose lower bounds above the ADI level when the level of
6907 confidence considered was 0.05, and only for the threshold of 360 when α was equal to 0.01. If the
6908 threshold considered is 240 μM and α is 0.05, the aspartame dose lower bound could be below the
6909 ADI level (in about 18 % of the times), for the case in which the confidence level considered was 0.01,
6910 then in 46.6% of the simulations, the aspartame dose lower bound was below the ADI level for the
6911 phenylalanine threshold of 240 μM and 5.1% for the phenylalanine threshold of 300 μM . It is
6912 important to highlight that for the heterozygote population, the results obtained should be interpreted
6913 with caution due to the limited number of doses found and the amount of individuals used to derive the
6914 summary data.

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6935 **L. ASSESSMENT OF BOTH THE VALIDITY AND RELIABILITY OF THE LONG-TERM STUDIES ON**
6936 **ASPARTAME AND DI-KETOPIPERAZINE BASED ON THE SUMMARIES OF THE UAREP**
6937 **REPORTS.**

6938 Only scientific issues have been considered in this examination of the UAREP reports (E102a, b, c).

6939 1. The Universities Associated for Research and Education in Pathology (UAREP) report consisted in
6940 an authentication review of selected materials submitted to the FDA relative to the application of
6941 Searle laboratories to market aspartame. It was issued on November 18 1978.

6942 From nearly 100 studies which Searle has presented to the FDA to market aspartame, 12 were selected
6943 by FDA and other parties for this review. They included:

6944 - five major studies of long term toxicity effects in the dogs, rats, and mice

6945 - two special neuropathology studies

6946 - the effects of aspartame in newborn rats

6947 - a two generation reproductive study in the rat

6948 - a series of screening tests for endocrinological and physiological responses

6949 - a report covering a few inconsequential observations on pregnant monkeys

6950 - a major study of the effects of aspartame and its metabolites in embryogenesis and
6951 teratogenesis in rabbits.

6952 E-28 106 week oral toxicity study in the dog. Forty Beagles (five / sex / treatment group) were fed
6953 aspartame daily in different dosages. Seven haematology, ten clinical chemistry and eight urinalysis
6954 parameters were measured periodically throughout the experiment. An additional ten chemical
6955 constituents were determined at 78 and/or 106 weeks. All the dogs were killed at 106 weeks and
6956 complete necropsies and histopathologic examinations performed.

6957 E-33,34, E-70, E-75 and E-76 share the objective of testing the effects of aspartame in life time
6958 toxicity studies. E-33,34 involved 440 rats in five groups with the highest dosage being 8 g/kg
6959 bw/day), E-70 involved 280 rats in control and two treatment groups. E-75 and E-76 utilized each 360
6960 mice, E-75 evaluated again aspartame whereas E-76 provided 3 graded dosages of its conversion
6961 product, diketopiperazine (76). The duration of the 4 experiments ranged from 103 to 108 weeks.
6962 Validation of the histopathology diagnosis was carried out on 35000 tissue sections from these 4
6963 experiments. As expected some discrepancies did exist. More than 99% of the slides were available
6964 for UAREP review.

6965 The discrepancies in diagnosis and the occasional missing slides had no predilection for any of the
6966 animal groups, or organ systems. UAREP' pathologists concluded that: certainly there was no
6967 evidence that either aspartame or DKP enhanced the production of tumours in these experiments.

6968 E-86 Supplemental study of dog brains from a 106 week oral toxicity study (E-28) and E-87
6969 Supplemental study of rat brains from 2 tumorigenicity studies (E-33,34 and E-70). Because of the
6970 possibility that there might be an increased incidence of brain tumours in dogs in study E-28 and in
6971 rats in studies E-33,34 and E-70, additional sections of brain were cut from the dog (E-86) and rats (E-
6972 86) and reviewed by Searle's neuropathology consultant Dr J.R.M. Innes. A panel of neuropathology
6973 experts convened by UAREP

6974 - agreed completely with Dr Innes that there were no brain tumors in the dogs (E-86)

6975 - generally agreed well with Dr Innes (E-86) for the rat studies. The 20 brain tumours
6976 diagnosed showed no statistically significant increase in any group when the tumours for the
6977 two experiments were combined. E-9 Toxicological evaluation of aspartame in the neonatal
6978 rat. Groups of five male and five female newborn rats were sacrificed at 5, 15, and 21 days
6979 with analysis of Five hematologic parameters and six chemical tests. The white blood cells
6980 counts were variable but statistically significantly depressed in the treatment groups. White
6981 blood counts were corrected for nucleated blood cells on the lab sheets but the uncorrected
6982 figures were used in the Entry report. This did not significantly affect the outcome of the
6983 statistical analysis. UAREP agreed with the EPL pathologic diagnostic of nuclear changes in
6984 renal tubular cells in the 15 and 21 day old rats, which apparently related to aspartame
6985 exposure. E-11 Two generation reproduction study in rats. This study was designed to
6986 characterize the effects of aspartame in the reproductive performance of the same strain of rats
6987 as used in other HLA-Searle experiments. UAREP agreed with the findings in E-11 report.
6988 Fewer discrepancies or problems were noted in this than in most of the other studies reviewed.
6989 E-19 Endocrine studies

6990 This report covers a battery of screening tests (7 hormone related tests and 6 physiologic responses
6991 tests) which were used by Searle for compound clinical testing. Some of these routine tests appeared
6992 to be carried out with less precision or documentation than some might desire. UAREP encountered
6993 some difficulty in reconstructing and interpreting the results of these routine tests

6994 E-88 Experiments in mated and pregnant Rhesus monkeys. Searle informed that the E-88 report was
6995 based on fragmentary data developed independently of Searle and without Searle's involvement in the
6996 design of the study. UAREP felt that this report was without design, inconsequential, and based on
6997 woefully inadequate and confusing data from an inadequate number of animals.

6998 E-90 An evaluation of the embryotoxic and teratogenic potential of aspartame in rabbits. These
6999 experiments involved 300 female rabbits and studied the effects of administering by gavage,
7000 aspartame and its metabolites, L-phenylalanine and L-aspartic acid, during fetal organogenesis in
7001 pregnant rabbits. The dosage level of administration was not equivalent to that recorded by the highest
7002 aspartame dose group. UAREP authenticated the findings in the skeletal system of the cleared whole
7003 fetuses. Seven of the 10 discrepancies UAREP found were minor malformations occurring in fetuses
7004 which were previously reported by Searle to have other malformations. Although it was not possible to
7005 examine the body cross-sections because of their altered conditions, UAREP felt that its validation of
7006 the skeletal specimens was adequate indication of the accuracy and significance of Searle findings.

7007 Overall, UAREP has interpreted the results only to the experiments as designed, it has addressed itself
7008 to the question of whether the experiments were carried out according to protocol plans and the
7009 accuracy and reliability with which the experiments were performed and reported to the FDA. At
7010 times, UAREP has commented on the interpretation of the significance of the data. Attempt was made
7011 to quantitate not only the number but the magnitude and significance of discrepancies and problems
7012 noted.

7013 There were many instances in which the earliest recorded data were not available. UAREP failed to
7014 agree with Searle and Hazleton on less than 1% of the computations and in most instances the
7015 differences were small in magnitude.

7016 For the histopathologic slides, the full information on which the original pathologist based his
7017 diagnosis was still available. Autolysis of tissues was present in both the rat and mouse slides from
7018 HLA. UAREP pathologists did not feel that it was sufficiently severe to materially interfere with
7019 making diagnoses on all but a few slides.

7020 Not only the agreement in diagnosis was generally good in the four larger studies but there was also
7021 good agreement in the smaller study in E-9.

7022 There was obviously considerable variation in the results within and between groups when measuring
7023 variability and statistical differences. Although a substantial number of minor and inconsequential
7024 discrepancies were noted in UAREP's validation studies, there were few if any, discrepancies which
7025 would produce a change of greater than 5% in the final numerical data being compared.

7026 The UAREP report finally stressed that the discrepancies it observed appeared randomly distributed
7027 between treated and control groups of animals.

7028 The Panel noted that UAREP and Bressler (1977) reported that in very many instances, the earliest
7029 original raw data were not available, some of the protocols appeared to be variable in time and fully
7030 documented only at termination of the study. They identified a lot of discrepancies and variability in
7031 the results of the studies under investigation. They underlined that:

- 7032
- 7033 • in all groups, many of the old animals were obviously in poor health and had tumours or
7034 advanced renal or liver diseases which, in the view of some of the UAREP investigators may
7035 have make it difficult to recognize a mild carcinogenic action of the study compound. In this
7036 connection limiting the experiment in time according to survival percentage was suggested.
 - 7037 • the development of standard operating procedures along with the non intentional use of ill
7038 animals (in some studies, several animals in both control and treated groups received
7039 potassium penicillin G due to unidentified infection diseases outbreaks) and together with the
7040 availability of the data and transparency of the methodology are fundamental requirements for
7041 a study to be fully reliable. All of these requirements were unfortunately not usual practice
7042 when these studies have been carried out.

7042 The Panel noted that, both reports did not conclude that the studies were not useful but that they
7043 were rather in line with the current practice in the early 70's before the development of the
7044 standardised procedures and Good Laboratory Practice (GLP); therefore their results should be
7045 considered. In addition, UAREP did not find evidence that the studies investigated were
7046 deliberately treated to produce biased results.

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