



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

9 October 2017
EMA/CHMP/134648/2015 corr. 1*
Committee for Medicinal Products for Human Use (CHMP)

Information for the package leaflet regarding aspartame and phenylalanine used as excipients in medicinal products for human use

Draft agreed by Excipients Drafting Group	19 April 2016
Adopted by CHMP for release for consultation	28 April 2016
Start of public consultation	4 May 2016
End of consultation (deadline for comments)	3 August 2016
Agreed by Excipients Drafting Group	8 November 2016
Adopted by CHMP	15 December 2016
Date of publication	9 October 2017

Keywords	<i>Excipients, Package leaflet, Aspartame, E 951, Phenylalanine</i>
-----------------	---

* Correction of an editorial mistake for phenylalanine. Please see the [corrected Annex](#) for further details.



Information for the package leaflet regarding aspartame and phenylalanine used as excipients in medicinal products for human use

Table of contents

Executive summary	3
Proposal for updated information in the package leaflet	4
Scientific background	5
1. Characteristics.....	5
1.1. Category (function)	5
1.2. Physico-chemical Properties.....	5
1.3. Use in medicinal products.....	5
2. Pharmaco-toxicological data	6
2.1. Toxicology	6
2.1.1. Aspartame.....	7
2.1.2. L-Phenylalanine.....	12
2.1.3. Methanol	13
2.1.4. L-Aspartic acid	15
2.1.5. 5-Benzyl-3,6-dioxo-2-piperazine acetic acid.....	16
2.2. Toxicokinetics	17
3. Clinical safety data	17
3.1. Safety in adult patients	17
3.2. Safety in special populations.....	18
4. Risk assessment	18
4.1. Mode of action Approach applied by EFSA for the food additive aspartame	18
4.2. Risk assessment on the use of aspartame in medicinal products.....	21
4.3. Risk Assessment of the impurity DKP.....	21
5. Safety information relevant for the package leaflet.....	22
Abbreviations	23
References – Bibliography.....	24
Annex 1 - Information in the package leaflet as per 2003 Guideline	26

Executive summary

This document has been written in the context of the revision of the Annex of the European Commission Guideline on 'Excipients in the label and package leaflet of medicinal products for human use' [2, 15].

Aspartame (L-aspartyl L-phenylalanine methylester) is an artificial sweetener used to substitute sugar in food and beverages and to make pharmaceuticals more palatable. It is nearly 180–200 times sweeter than sucrose [18].

Aspartame is hydrolysed in the gastrointestinal tract into aspartyl phenylalanine and methanol. Further hydrolysis of aspartyl phenylalanine to aspartic acid and the essential amino acid, L-phenylalanine, produces a risk for patients with the homozygous gene for phenylketonuria [27]. Therefore, according to the Guideline on 'Excipients in the label and package leaflet of medicinal products for human use' (CPMP/463/00 Rev. 1) dated 2003 [15] warnings for patients with phenylketonuria with a zero-threshold for use have to be given in the package leaflet of aspartame containing medicinal products: "Contains a source of phenylalanine. May be harmful for people with phenylketonuria."

The hydrolysis product of aspartame, L-phenylalanine, is also used as excipient mainly to stabilise certain protein solutions. Therefore, a similar warning statement like for aspartame is required for L-phenylalanine containing medicinal products [15].

The main reason for re-assessing the information on the package leaflet was to review its use as sweetener in paediatrics. Simultaneously, EFSA was starting a re-evaluation of the safety of aspartame as a food additive in the EU (E 951) which was published in December 2013 [12]. The EFSA assessment served as the basis for the present evaluation of aspartame as excipient in medicinal products.

The EFSA ANS Panel confirmed the acceptable daily intake (ADI) value of 40 mg/kg bw/day formerly established by JECFA (Joint FAO/WHO Expert Committee on Food Additives) and the SCF (Scientific Committee on Food) for aspartame.

As a conclusion of the actual review on aspartame, no essential changes are proposed for the package leaflet of medical products. ADI values established by EFSA, however, do not apply to infants below 12 weeks of age [10]. A corresponding statement is included in the "comments" column for the benefit of applicants and the competent authorities.

Proposal for updated information in the package leaflet

Name	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Aspartame (E 951)	Oral	Zero	<p>This medicine contains x mg aspartame in each <dosage unit><unit volume> <which is equivalent to x mg/<weight><volume>>.</p> <p>Aspartame is a source of phenylalanine. It may be harmful if you have phenylketonuria (PKU), a rare genetic disorder in which phenylalanine builds up because the body cannot remove it properly.</p>	<p>Aspartame is hydrolysed in the gastrointestinal tract when orally ingested. One of the major hydrolysis products is phenylalanine.</p> <p>Information to consider for the SmPC: Neither non-clinical nor clinical data are available to assess aspartame use in infants below 12 weeks of age.</p>

Name	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Phenylalanine	All	Zero	<p>This medicine contains x mg phenylalanine in each <dosage unit><unit volume> <which is equivalent to x mg/<weight><volume>>.</p> <p>Phenylalanine may be harmful if you have phenylketonuria (PKU), a rare genetic disorder in which phenylalanine builds up because the body cannot remove it properly.</p>	

Scientific background

1. Characteristics

1.1. Category (function)

Aspartame is used as sweetening agent in oral pharmaceutical formulations, beverages and food products.

1.2. Physico-chemical Properties

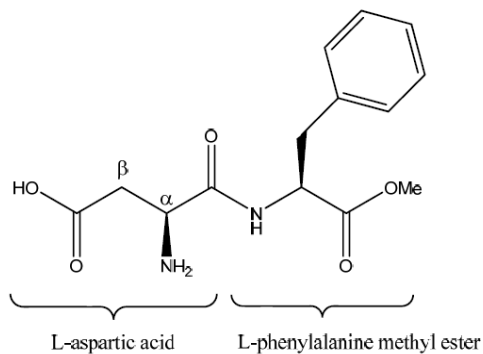


Fig.1 Structural formula of α-aspartame

Chemical formula: C₁₄H₁₈N₂O₅

Codes:

Ph.Eur. Aspartame

(3S)-3-Amino-4-[[[(2S)-1-methoxy-1-oxo-3-phenylpropan-2-yl]amino]-4-oxobutanoic acid (methyl α-l-aspartyl-l-phenylalaninate).

USP/NF: Aspartame

CAS Registry Number [22839-47-0]

Molecular weight: 294.31 g/mol

Solubility: sparingly soluble or slightly soluble in water and in ethanol (96%), practically insoluble in hexane and in methylene chloride.

1.3. Use in medicinal products

Aspartame is used as an excipient in oral preparations. Aspartame content of medicinal products is usually below 100 mg as at medicinal product. Only a few of those products are indicated for children less than 12 years of age. Examples of the latter are amoxicillin or ibuprofen containing effervescent tablets or bacterial suspensions to treat diarrhoea in sucklings and infants. Aspartame levels of more than 100 mg, resulting in daily aspartame doses of more than 2000 mg, are typically found in medicinal products indicated for short term use such as laxatives in adults or children older than 12 years of age.

2. Pharmacotoxicological data

The evaluation of the pharmacotoxicological data and animal pharmacokinetics is mainly based on the scientific opinion on aspartame recently published by the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) [12]. Although EFSA's focus is on aspartame when used as a food additive, the data material considered for evaluation and the kind of assessment is quite similar to what is applied for excipients in pharmaceuticals.

The EFSA panel performed a thorough assessment on the basis of an enormous amount of data. Original reports, previous evaluations, additional literature and data made available following a public call were evaluated. Data rejected by EFSA for evaluation because of flaws in experimental designs, are generally not considered in the current review. EFSA confirmed the former ADI value of aspartame of 40 mg/kg bw/day.

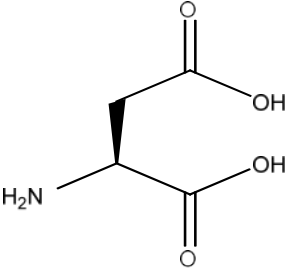
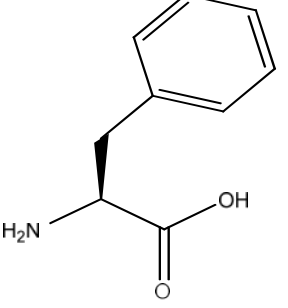
2.1. Toxicology

Studies in animals and humans have shown that aspartame (α -aspartame) is fully hydrolysed in the gastrointestinal tract when orally ingested. The major hydrolysis products of aspartame are L-phenylalanine, L-aspartic acid and methanol.

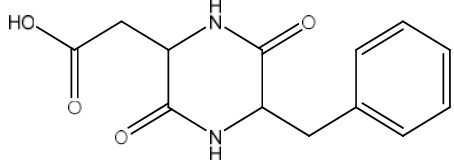
The degradation products 5-benzyl-3,6-dioxo-2-piperazine acetic acid (DKP) and β -aspartame may also be present as impurities. β -Aspartame is the non-sweet isomer of α -aspartame.

Therefore, aspartame as well as its hydrolysis products and the main degradation product DKP were separately evaluated by EFSA.

Major hydrolysis products of aspartame

Methanol	CH ₃ OH Molecular weight: 32.04 g/mol
L-Aspartic acid (= aspartic acid in the present review)	 Molecular weight: 133.11 g/mol
L-Phenylalanine (=phenylalanine in the present review)	 Molecular weight: 165.19 g/mol

Major degradation product of aspartame

5-Benzyl-3,6-dioxo-2-piperazine acetic acid (DKP)	 <p data-bbox="799 405 1198 434">Molecular Weight: 262.26 g/mol</p>
---	---

Tab. 1: Hydrolysis and degradation products of aspartame

2.1.1. Aspartame

In various animal species, including mouse, rat, rabbit, dog and monkey, the absorption, distribution, metabolism and excretion of aspartame have been investigated using different radiolabelled forms of ¹⁴C-aspartame. Radioactivity associated with unchanged aspartame was not detectable in plasma of experimental animals. Based on the sensitivity of the detection of radioactivity following labelling of the phenylalanine, aspartate and methyl moieties of aspartame, it can be concluded that aspartame is completely hydrolysed in the gut to yield aspartate, phenylalanine and methanol. These metabolites are then absorbed and enter normal endogenous metabolic pathways.

Single dose toxicity

The acute toxicity of aspartame was studied in mice, rats and rabbits with oral doses up to 5000 mg/kg bw or intraperitoneal (IP) injections of up to 1000 mg/kg bw. LD₅₀ (lethal dose) values were in excess of the highest doses administered to each species.

Rats or dogs receiving 100 mg/kg bw aspartame by intravenous (IV) injection did not show any significant changes in histopathology or haematology, clinical chemistry and urine analysis parameters evaluated, apart from phlebitis at the site of implantation of the cannula in all rats.

Short-term and sub-chronic toxicity

Rats or mice were administered doses of up to 13000 mg/kg bw/day aspartame via diet for 4 weeks. No treatment-related changes were reported except for a heavily coated intestinal mucosa with a clear, moderately viscous fluid at the highest dosage levels.

In one study with 8 weeks treatment of rats via diet, males showed a significantly higher liver to body weight ratio at the highest dose of 125 mg/kg bw/day. No treatment-related changes were reported in haematology or urine analysis parameters. Weanling rats (23 days of age at begin of study) received an aspartame containing diet for 9 weeks. Animals either received a basal diet or a diet containing 9% w/w aspartame (on average 12.2 mg/kg day) or 5% w/w phenylalanine (on average 6.5 mg/kg day). Decreasing mean daily dosage levels were consumed from 24 to 7 mg/kg aspartame and 13 to 4 mg/kg phenylalanine. Reduced body weight gain correlating with a reduced absolute feed consumption was the only finding reported. No significant treatment-related changes in haematology, clinical chemistry, urinalysis, relative organ weights or pathology were seen. After treatment of dogs with aspartame containing gelatine capsules for 8 weeks, no changes were observed on haematological, clinical chemistry and pathology parameters for doses up to 125 mg/kg bw/day.

Chronic toxicity and carcinogenicity

Aspartame (DKP content of 0.8% to 1.2%) was administered in the diet to male and female mice amounting to dose level of 0, 1000, 2000, 4000 mg/kg/day for 104 weeks. Mice were approximately 28 days of age at start of treatment. No adverse effects regarding to survival rate, incidence of

neoplasms and non-neoplastic changes in any organs or tissues were observed. The NOAEL (no observed adverse effect level) was established at 4000 mg/kg bw/day, the highest dose tested.

Rats were administered different doses of aspartame (DKP content varied from 0% to 1.5%) via diet for 104 weeks post-weaning. No adverse effects or an increased incidence of neoplasms or non-neoplastic lesions except for renal changes in males at 8000 mg/kg bw/day were observed. The NOAEL was identified at 4000 mg/kg bw/day.

In another chronic toxicity study in rats, aspartame (DKP content of 0.8% to 1.2%) administered via diet exhibited no adverse effects regarding neoplastic or non-neoplastic changes. Treated rats were obtained as F1 weanlings from parental animals which had been pre-treated with the same aspartame doses 60 days prior to mating throughout mating, gestation and nursing. The NOAEL was established at 4000 mg/kg bw/day, the highest dose level studied.

Supplemental histopathological examinations of all brains were performed for both carcinogenicity/chronic toxicity studies in the rat. Since no dose-relationship of brain tumours was observed and the incidence of tumours lay within background controls, it was concluded that evidence of intracranial tumourigenic effect was not demonstrated.

An additional 2-year chronic toxicity study was performed in rats with aspartame (DKP content up to 1%) in the diet reaching dose levels of up to 4000 mg/kg bw/day. An additional group was fed 4000 mg/kg bw/day aspartame plus DKP (3:1). There was no evidence of a treatment-related increase in neoplastic or non-neoplastic lesions which was confirmed in a second study re-evaluating the non-neoplastic findings. A dose-dependent increase in focal mineralisation of the renal pelvis was observed with aspartame in both, males and females. Mineralisation of the kidney probably due to mineral imbalance is a common finding in rats [19]. Therefore, the NOAEL was established at 4000 mg/kg bw/day aspartame. Lack of toxicity of the combination of aspartame with DKP was also shown.

Two long-term carcinogenicity studies in rats [24, 25] and one in mice [26] have been performed by the European Ramazzini Foundation. In the rat studies, animals were exposed from 80 up to 100 000 mg aspartame/kg in the diet, equivalent to 4 up to 5000 mg aspartame/kg bw/day. A range of inflammatory changes were observed in different organs of the rats particularly in the lungs and kidneys. An increased incidence of neoplastic changes, among others lymphomas/leukaemias, malignant schwannomas of the peripheral nerves and mammary gland carcinomas were reported. In the mouse study, Swiss mice were treated from prenatal life (12 days of gestation) until death with aspartame in the feed at doses from 2000 to 32000 mg/kg feed, equivalent to 250 or 4000 mg/kg bw/day, respectively. In male mice the incidence in hepatocellular carcinomas and alveolar/bronchiolar carcinomas was increased.

All three studies were evaluated by the former AFC Panel, the ANS Panel of EFSA (See the EFSA report 2013 [12], page 70–71). The Panels considered the studies would not contribute to the further assessment of the carcinogenic risk of aspartame since they either had deficits questioning the validity of findings or tumours were observed within background ranges. In particular, the high background incidence of chronic inflammatory changes in the lungs and other vital organs and tissues, and the uncertainty regarding the correctness of the diagnoses of some tumour types were major confounding factors in the interpretation of the findings of the rat study. The ANS Panel also noted that the increase in the incidence of mammary carcinomas was not considered indicative of a carcinogenic potential of aspartame since the incidence of mammary tumours in female rats is rather high and varies considerably between carcinogenicity studies. The only consistent findings reported by the authors in the two rat studies were an increased incidence of lymphomas/leukaemias in female rats and an increased incidence of lymphomas/leukaemias in treated males and females of the high dose group. A

full evaluation of these studies, including an assessment of the malignant schwannomas and the hyperplastic and neoplastic lesions of the epithelium of the renal pelvis, ureter and urinary bladder, has been published elsewhere [6, 7, 8].

Concerning the carcinogenicity study in Swiss mice, the ANS Panel (EFSA ANS Panel, 2011) and EFSA [9] concluded that the hepatic and pulmonary tumour incidences reported by Soffritti et al. 2010 [26] all fall within their own historical control ranges for spontaneous tumours. It was also noted that Swiss mice are known to have a high background incidence of spontaneous hepatic and pulmonary tumours.

The US National Toxicology program performed several 9-month carcinogenicity studies in genetically modified mouse models with oral doses up to 7660 and 9620 mg/kg bw/day in males and in females, respectively. Altogether, there was no evidence of treatment-related neoplastic or non-neoplastic lesions in any of these studies.

5-month old Beagle dogs were exposed to aspartame (DKP content up to 1%) via dietary administration for 106 weeks at dose levels up to 4000 mg/kg bw/day. No meaningful alterations in body weight, feed consumption, physical examination, clinical chemistry examinations or gross and microscopic findings were observed. An extended histopathological examination of the brain tissue did not reveal any lesions. The NOAEL in this study was the highest dose of 4000 mg/kg bw/day.

In a chronic toxicity study in monkeys, grand mal seizures were observed in animals treated with doses above 3000 mg/kg bw/day via diet. However, the significance of this finding is not clear, since all animals were also affected by a Shigella infection during the study.

Genotoxicity

Aspartame was investigated for mutagenicity in several Salmonella typhimurium strains with and without metabolic activation for doses up to 10000 µg / plate. With the limitation that some tester strains were not studied, altogether aspartame did not show mutagenicity in bacterial systems. DNA damaging activity of aspartame was tested negative in the in vitro primary rat hepatocyte/DNA repair assay at concentrations of 5 and 10 mM. Concerning mammalian systems in vitro, no conclusion could be drawn on the gene and chromosomal levels because those endpoints were not studied.

Aspartame was tested negative in two dominant lethal tests in rats at doses up to 2000 mg/kg bw/day. Bone marrow cells were evaluated for chromosome aberrations after treatment of albino rats with doses of up to 4000 mg/kg bw/day for five days. An acute bone marrow micronucleus test was also conducted in rats with aspartame doses up to 2000 mg/kg bw/day. Aspartame tested negative in both in vivo tests. Peripheral blood micronucleus tests were performed in male and female transgenic mice. Equivocal positive findings were only described in female p53 haplosufficient mice but not in males after 9 month treatment with 8180 mg/kg bw/day; in two other mouse strains the results were negative. Aspartame did not induce any significant increases in DNA migration in a Comet assay conducted in mice, where analyses were performed in the stomach, colon, liver, kidney, bladder, lung, brain and bone marrow.

Reproductive and Developmental Toxicity

Rat / Fertility

In a two-generation reproduction toxicity study performed in rats with aspartame applied in the diet the NOAEL was identified at 2000 mg/kg bw/day, based on lower pup weights at weaning in both generations. A fertility study was conducted in male and female rats with oral application of

aspartame. Aspartame (with a DKP content of 0.3%) had no significant effect on maternal sexual behaviour or fertility and a NOAEL of 4000 mg/kg bw/day was established.

In a different study (Brunner et al. 1979) [5] aspartame was administered via the diet during pre-breeding, gestation, lactation and post-weaning. Aspartame doses in the highest dose group varied between 5000 and 9600 mg/kg bw/day. Rats of the highest dose group lost more weight than other dose groups during lactation and showed an increased offspring mortality. Also pups fed the highest aspartame dose weighed significantly less than controls after 30 days and remained lighter throughout the study. Eye opening was delayed in pups from the highest dose group whereas timing of pinnae detachment and incisor eruption were not affected.

Aspartame was administered in drinking water to rats from day 12 prior to conception until the pups were 38 days old (after weaning pups were given the same treatment like adult rats). Different tests performed showed that aspartame exposure did not affect reflex development, morphological development and spatial memory of pups at doses up to 1614 mg/kg bw/day given to adult rats or 3566 mg/kg bw/day given to pups later on.

Mice / Embryo-fetal development

Aspartame (DKP content 0.29%) was administered to pregnant mice in the diet from gestation day (GD) 6 to 15. No treatment related effects were observed on maternal and fetal parameters investigated (e.g. maternal conception rates and body weight, fetal abnormalities). The NOAEL was established at the highest dose of 5700 mg/kg bw/day.

Pregnant mice were administered aspartame by gavage from GD 15 to 18. In utero exposure to aspartame did not affect physical and functional development of the visual system of the pups at doses up to 4000 mg/kg bw/day

Rat / Embryo-fetal development

An embryotoxicity study was performed with dietary administration of aspartame from GD 6 to 15. No maternal toxic effects or evidence of treatment related feto-pathological effects were observed in treated litters. The NOAEL was identified at the highest dose of 4000 mg/kg bw/day.

Rabbit / Embryo-fetal development

In the rabbit, several studies were performed with aspartame administration in the diet or by gavage during different phases of gestation. When aspartame was applied in the diet, feed intake was often remarkably decreased in high dose groups so that actual aspartame doses were comparable in the low and high dose aspartame groups. Reduced food consumption was frequently accompanied by lower maternal body weights which resulted in abortions or effects on skeletal development of fetuses. Altogether, results of the studies on embryo-fetal development in the rabbits indicate a highest NOAEL of 2400 mg/kg bw/day administered in the diet.

One study performed with gavage application used additional groups receiving phenylalanine (820 mg/kg bw/day) and aspartic acid (1100 mg/kg bw/day), which, on a molar basis, were levels equivalent to 75% and 134% of the amount of the same amino acids theoretically available from 2000 mg/kg bw/day aspartame. Reduced food consumption resulting in body weight loss, reduced fetal weights, increases in malformations and abortions were observed for the high dose aspartame (2000 mg/kg bw/day) and the phenylalanine group. Accordingly, the NOAEL of the study in rabbits dosed by gavage was 1000 mg/kg bw/day based on maternal toxicity in the 2000 mg/kg bw/day group which was accompanied by a severe decrease in feed intake and developmental toxicity (weight loss and malformations).

High levels of aspartame-derived phenylalanine may partially be responsible for effects observed in the embryo-fetal development studies with aspartame, because similar effects were seen when pregnant rabbits were treated with phenylalanine.

Toxicokinetics in pregnant rabbits

Pregnant rabbits were fed an aspartame containing diet 6% from GD 6 to GD 20 [21]. Phenylalanine and tyrosine content of maternal and fetal body fluids were investigated. Maternal plasma phenylalanine and tyrosine levels significantly increased in aspartame-fed animals compared to controls reaching a peak on GD 9 (phenylalanine 296.6 μM ; tyrosine 546 μM) and returned to normal on GD 20. Fetal plasma tyrosine was significantly higher in aspartame-fed dams with a peak on GD 20 (408 μM) whereas there was no change in phenylalanine levels. Phenylalanine and tyrosine concentrations in the amniotic fluid were consistently higher in treated animals compared to controls, with peaks on GD 20 (phenylalanine 1235 μM , tyrosine 1843 μM).

Rat / Pre-postnatal development

After treatment during gestation and lactation with 2000 and 4000 mg/kg bw/day aspartame in the diet (DKP content of 0.5%), the number of viable pups per litter and pup survival until weaning was significantly decreased in the high dose group and a decrease in maternal body weights at postpartum day 21 was observed. Therefore, the NOAEL was identified at 2000 mg/kg bw/day. In two further studies on pre-postnatal development significant body weight suppression and a decrease in survival at weaning and incomplete eyelid opening was observed for pups from aspartame (DKP content 0.2 and 0.5%, respectively) treated dams. Mothers showed decreased feed intake and a reduced body weight resulting in a NOAEL of 2000 mg/kg bw/day.

An additional study compared the effect of 4000 mg/kg bw/day aspartame (DKP content 0.5%) in the diet with those of L-phenylalanine (1800 mg/kg bw/day), L-aspartic acid (1700 mg/kg bw/day) or L-phenylalanine plus L-aspartic acid (2100 and 1800 mg/kg bw/day) on pre-postnatal development. L-Phenylalanine on its own or in combination with aspartic acid as well as aspartame decreased maternal and pup body weight. Only for the combination of L-phenylalanine and L-aspartic acid also pup survival was significantly lower compared to controls. It was discussed whether effects observed with aspartame might be due to aspartame derived phenylalanine.

After delivery of pups, rat dams were fed aspartame in the diet during the whole lactation period (for 21 days). Reduced feed consumption accompanied by a loss in body weight was observed in dams of high dose groups. Pup body weight and survival was also reduced. A higher incidence of resting or inactive mammary glands was observed in high dose dams attributed to a lack of suckling pups and severe feed restriction. The NOAEL was identified at 7120 mg/kg bw/day.

Monkey / Postnatal development

In a postnatal development study aspartame was fed to monkeys for 9 months. No clinical signs and no effects on haematological, plasma chemistry or urine analysis parameters were observed. Aspartame had no impact on learning performance and gearing ability. The NOAEL was established at the highest dose of 2500–2700 mg/kg bw/day.

Neurotoxicity

Weanling rats were given a standard diet (controls) or a standard diet either supplemented with aspartame or phenylalanine for 13 weeks. No differences in the physical condition were noted between the groups. For both high treatment groups (6000 mg/kg bw/day phenylalanine or aspartame)

statistically significant impaired learning performances were reported. At a lower dose of phenylalanine (3000 mg/kg bw/day), the effects were not reported.

The EFSA Panel agreed with former Scientific Committee on Food (SCF and EFSA Advisory Forum opinions that no new data are available which report a link between aspartame consumption and enhanced susceptibility to seizures, behaviour, mood and cognitive function.

Summary on aspartame

Aspartame showed a very low acute toxicity. Similarly, sub-acute and sub-chronic studies did not indicate any significant toxic effects in rats, mice and dogs.

Overall, the available data do not show a genotoxic concern for aspartame.

The NOAEL for the long-term toxicity and carcinogenicity studies in rodents was 4000 mg/kg bw/day. Lack of toxicity was also demonstrated for 4000 mg/kg bw/day aspartame plus DKP (3: 1). Beagle dogs exposed to aspartame for 106 weeks also showed a NOAEL of 4000 mg/kg bw/day.

In the reproductive and developmental toxicity studies conducted in rodents, the lowest NOAEL identified in rats was 2000 mg/kg bw/day and 4000 mg/kg bw/day in mice. In rabbits, a NOAEL of 2400 mg/kg bw/day was established for aspartame administered in the diet and 1000 mg/kg bw/day for aspartame administered by gavage. One study in the monkey performed with emphasis on effects of aspartame on the postnatal development showed a NOAEL of 2500 mg/kg bw/day.

2.1.2. L-Phenylalanine

Phenylalanine exists as D and L-enantiomers, and L phenylalanine is an essential amino acid required for protein synthesis. L- Phenylalanine is hydrolysed by phenylalanine hydrolase (PAH) to tyrosine by a reaction requiring molecular oxygen and the cofactor tetrahydrobiopterin (THB).

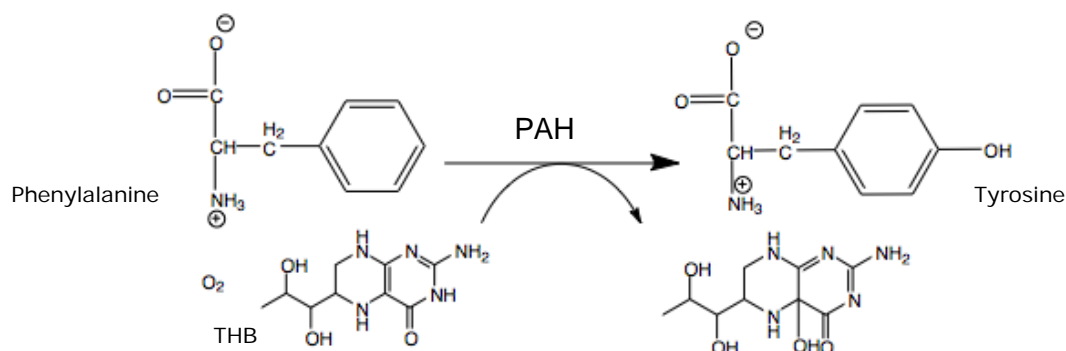


Fig.2: Hydrolysis of L-phenylalanine to tyrosine

Human Data

Mutations in the PAH gene result in phenylketonuria (PKU), an autosomal recessive inborn error of L-phenylalanine metabolism causing hyperphenylalaninaemia. Untreated PKU is associated with an abnormal phenotype including growth failure, microcephaly, seizures and intellectual impairment caused by the accumulation of L-phenylalanine and its by-products. High phenylalanine levels are neurotoxic, mainly due to its inhibitory action on the transport of free L-amino acids, necessary for protein and neurotransmitter synthesis. Hyperphenylalaninaemia results from a number of different mutations within the PAH allele producing a spectrum of phenotypes including classic PKU, moderate

PKU, mild PKU and mild hyperphenylalanaemia. For all these PKU phenotypes, plasma phenylalanine levels are above 600 μM when on an unrestricted diet. According to the NIH (US National Institutes of Health) Consensus Statement on PKU, it is assumed that levels in excess of dietary requirement for this essential amino acid but below 600 μM do not lead to brain damage. NIH also published reference values for different population groups discussed below.

Altogether human data on phenylalanine pharmacokinetics available from PKU patients were the basis for EFSA's risk assessment of aspartame.

Animal data

Many studies have been performed to reproduce the biochemical and behavioural effects observed in PKU patients by administration of large amounts of phenylalanine to different animal species. Rats showed a decrease in growth rate when fed large amounts of phenylalanine. In pregnant rabbits reduced food consumption resulting in body weight loss, decreases in fetal weights and malformations of fetuses were observed with phenylalanine. Decreases in maternal and pup weight were noted in pre-postnatal development study with phenylalanine in rats. (For reproductive toxicity of phenylalanine please refer to the respective aspartame section above.)

PKU was produced in monkeys by feeding six infant Rhesus monkeys 3000 mg/kg bw/day phenylalanine soon after birth and for up to 3 years. Monkeys showed elevated plasma levels of phenylalanine. Grand mal convulsions like those observed in some children with PKU were also observed in experimental animals as well as intellectual deficits.

2.1.3. Methanol

After oral administration methanol is stepwise oxidised to formaldehyde and then to formate. Formate is finally metabolised to carbon dioxide via different pathways. Formaldehyde and formate can also enter the one carbon metabolic pool through tetrahydrofolic acid and may from there contribute to biosynthesis of purines and pyrimidines.

Single dose toxicity

The LD₅₀ reported for methanol was 7300 mg/kg bw in mice, 5628 mg/kg bw in rats and 7000 mg/kg bw in monkeys.

Subchronic-toxicity

In a 90-day study in rats with oral application of methanol a NOAEL of 500 mg/kg bw/day was identified. Increases in serum alanine transaminase and alkaline phosphatase as well as decreases in blood urea nitrogen and mean corpuscular haemoglobin and cell volume were observed in the high dose group of 2500 mg/kg bw /day. A higher incidence of colloid in the hypophyseal cleft of the pituitary gland was also observed in the high dose group.

Chronic toxicity and carcinogenicity

Two studies on chronic toxicity and carcinogenicity are available with oral application of methanol: Apaja 1980 [3] and Soffritti et al. 2002 [23]. However, EFSA considered both studies not suitable for cancer risk assessment either because of poor experimental design or doubtful validity of the conclusions.

Genotoxicity

In vitro, a negative bacterial reverse mutation test, a negative gene mutation test with *Schizosaccharomyces pombe* and a negative prophage induction test with *Escherichia coli* WP2(λ) with and without metabolic activation were observed. Concerning relevant in vivo studies, methanol did not induce micronuclei in mice given oral doses of up to 8410 mg/kg bw. Furthermore, no increase in sex-linked lethal mutations in either the wild type or basic strain of *Drosophila melanogaster* was found after 1000 mM methanol feeding. In addition, in studies following intra-peritoneal application or inhalation exposure of mice, rabbits or monkeys, no evidence of induction of DNA-damage (8-oxodG) formation, micronuclei and SCE was observed.

Overall, the available data do not indicate a genotoxic concern for methanol.

Reproductive and Developmental Toxicity

Most studies available for reproductive toxicology of methanol were performed with inhalation exposure. These studies were considered for risk assessment using respective models to convert inhalation exposure to exposure per kg body weight.

Fertility

Female macaque monkeys were exposed up to 1800 ppm (approximately 185 mg/kg bw/day) methanol vapour for 2.5 hours for 7 days a week prior to breeding and throughout pregnancy (~120 days). A significant but not dose-related reduction in the mean length of pregnancy was observed; otherwise mothers remained healthy during the study. No methanol-related effects were apparent on the birth weight or health of the offspring.

Embryo-fetal development

Mice were administered doses of up to 15000 ppm methanol by inhalation for 7 hours/day or 4000 mg/kg bw/day by oral gavage from GD6-15. After inhalation exposure, increased incidences of exencephaly and cleft palate, embryo-fetal death, low fetal weights, complete litter resorptions and cervical ribs were observed in treated animals and a NOAEC of 1000 ppm methanol was established. After oral exposure to 4g daily, plasma methanol levels during pregnancy were comparable to the 10000 ppm inhalation exposure group. Incidences of resorptions, external defects and decreases in fetal weights were similar to those found in the 10000 ppm inhalation exposure group. The NOAEC of 1000 ppm identified in the inhalation study equals an oral dose of approximately 560 mg/kg bw/day (calculation according to the method of Alexander et al. 2008 [1]).

Two studies in mice investigated the influence of dietary folic acid on the developmental toxicity of methanol using high doses (4 and 5 mg/kg bw/day) of methanol. Developmental effects observed (e.g. cleft palate, exencephaly) can be attributed to methanol but no clear answer could be given whether effects were enhanced by folic acid deficiency.

Treatment related malformations, predominantly extra or rudimentary cervical ribs, urinary and cardiovascular defects, and maternal toxicity was seen in fetuses of rats exposed 7 hours/day to 20000 ppm methanol by inhalation during GD7-15. A NOAEC (no observed adverse effect concentration) of 5000 ppm equalling an approximate NOAEL of 2070 mg/kg bw/day (calculated according to Alexander et al. 2008 [1]) was established.

Summary on methanol

LD₅₀ values of more than 5000 mg/kg have been reported for methanol. A sub-chronic toxicity study in rats resulted in an oral NOAEL of 500 mg/kg bw/day. The data set on genotoxicity is limited but

overall, in vitro and in vivo data do not indicate a genotoxic concern for methanol. No adequate long-term toxicity or carcinogenicity studies are available for methanol. Similarly no adequate studies exist for effects of methanol on fertility and reproductive performance as well as pre-postnatal development. Developmental toxicity was investigated with inhalation exposure in mice and rats. The calculated NOAEL for mice was approximately 560 mg/kg bw/day and for rats 2070 mg/kg bw/day.

The metabolite of methanol, formaldehyde, is classified by IARC (International Agency for Research on Cancer) as a known human and animal carcinogen that causes nasopharyngeal cancer in humans and squamous cell carcinomas in the nasal passages of rats. The ANS Panel of EFSA therefore also performed a risk assessment on formaldehyde derived from aspartame. The Panel calculated a plasma steady state concentration of 12 µM methanol that could arise maximally from aspartame derived methanol at the current ADI of aspartame and a peak concentration of 60 µM methanol after three divided doses of aspartame at the current ADI (for details refer to [11]). Methanol is oxidised to formaldehyde and a concentration of 12 µM methanol will produce at most, a steady state concentration of 12 µM formaldehyde/formaldehyde acetal (structure of formaldehyde present at tissue level). It was concluded that the increase in formaldehyde acetal at the current ADI of aspartame with an assumed 100% conversion from aspartame to formaldehyde would be less than 3% (for steady state) and less than 15% (for peak levels) of normal intracellular endogenous levels. A maximum amount of 2310 mg aspartame per day is found for one medicinal product in Germany which is indicated for single use in adults or children over 12 years. This would result in an exposure of approximately 53 mg/kg bw aspartame for a 12 year old child with a body weight of 43 kg [11] and is in the range of the ADI. According to the EFSA assessment, formaldehyde levels resulting from aspartame will still be below steady state as well as peak endogenous formaldehyde levels even for this medicinal product with the highest content of aspartame. Accordingly, a carcinogenic risk through formaldehyde exposure by the intake of an aspartame containing medicinal products can be excluded.

2.1.4. L-Aspartic acid

L-Aspartic acid, a non-essential amino acid, is among the most common amino acids found in the diet. The metabolism of orally administered L-aspartic acid has been studied in several species. L-aspartic acid is rapidly and to a high level eliminated as CO₂ which seems to result from both the decarboxylation of L-aspartate to alanine and CO₂ and the transamination reaction with pyruvate to form alanine and oxaloacetate in the intestinal mucosal cells. The alanine and oxaloacetate formed then enter the tricarboxylic acid cycle to eventually form CO₂. The remaining aspartic acid seems to be incorporated into normal body constituents or used for gluconeogenesis.

Animal data

L-Aspartic acid is an excitatory neurotransmitter of the central nervous system. Excitatory amino acids are known to cause neurodegeneration when nerve cells expressing glutamate receptors become overstimulated following exposure to excessive levels of glutamate and L-aspartate. However, kinetic studies on aspartame have shown that plasma L-aspartate levels do not substantially change following bolus or repeated administration of aspartame.

A NOAEL of 1100 mg/kg bw/day was identified for aspartic acid in an embryo-fetal development study conducted in rabbits (refer to section on reproductive toxicity of aspartame). Aspartate and glutamate can cause hypothalamic neuronal death in neonatal rodents, if given orally in large doses of 500 mg/kg bw or higher to juvenile animals. Mouse pups were found to be the most sensitive animals, e.g. in one study dose-dependent hypothalamic neuronal necrosis was found with 650 mg/kg bw doses and higher.

In a 90-day feeding study in rats, no signs and symptoms of neurotoxicity were observed at L-aspartic acid doses as high as 2965.9 mg/kg bw/day for females and 2770.2 mg/kg bw/day for males. A NOAEL of approximately 700 mg/kg bw/day for males was identified based on an apparent dose-dependent regenerative tubules degeneration accompanied by inflammatory cell infiltration.

2.1.5. 5-Benzyl-3,6-dioxo-2-piperazine acetic acid

The toxicokinetic properties of DKP have been studied in rat, rabbit, monkey and man. DKP was not well absorbed from the gastrointestinal tract and was primarily recovered as unchanged DKP in the faeces. Following oral administration, DKP is assumed to be metabolised to phenylacetic acid by the bacterial metabolism. Phenyl acetic acid can then be absorbed and rapidly excreted in the urine as phenyl acetic acid and following conjugation with glutamate as phenylacetylglutamine.

Phenylacetamine accounts for 20% (rat) to 50% (monkey and man) of the orally administered DKP.

Plasma and urinary concentrations of DKP were measured in normal adult subjects ingesting 2.2 mg DKP/kg as part of a dose of 200 mg aspartame/kg bw. DKP plasma concentrations were always below the detection limit (less than 1 µg/mL). Mean total urinary DKP excreted during the first 24-hour period after dosing was 6.68 ± 1.30 mg (corresponding to 4.83% of the ingested DKP dose) with 44% of total urinary excretion occurring in the first 4 hours after dosing.

Single-dose toxicity

The acute toxicity of DKP was studied in mice, rats and rabbits at oral doses up to 5000 mg/kg bw. No remarkable motor or behavioural effects or mortalities were noted.

Subchronic toxicity

Mice or rats administered 1000 mg/kg bw/day DKP by gavage for two weeks did not show any treatment-related changes in haematology, clinical chemistry, urinalysis or histopathology. Rats were fed DKP for 5 weeks in the diet at dose levels up to 6000 mg/kg bw/day. There were no treatment-related changes in haematology, clinical chemistry, urinalysis or histopathology. Female rats in the high dose group showed a decrease in body weight resulting from a decrease in feed intake.

Genotoxicity

Although test systems exhibited some limitations like the absence of some test strains, altogether DKP was not mutagenic in bacterial systems. The in vivo genotoxicity studies comprised chromosome aberration in bone marrow erythrocytes and dominant lethal assays both of which were negative. In conclusion, the data on genotoxicity do not indicate a genotoxic potential of DKP.

Chronic toxicity and carcinogenicity

The chronic toxicity of DKP was studied in mice and rats. DKP doses of up to 1000 mg/kg bw/day were administered in the diet to mice for 110 weeks. There was no effect on the physical appearance and behaviour of animals and no consistent treatment-related haematology/clinical chemistry and urinalysis findings were observed. There was no evidence of an effect with respect to neoplastic or non-neoplastic changes. A NOAEL of 1000 mg/kg bw/day was established, the highest dose tested.

Rats administered DKP doses of up to 3000 mg/kg bw/day in the diet for 115 weeks showed no effects regarding survival rate, body weight gain, physical examination and haematology findings, and incidence of neoplasms or non-neoplastic changes in any organ or tissue. DKP produced a significant decrease in urinary pH in high dose females which was attributed to acidic metabolites of DKP in the urine. A decrease in serum cholesterol was also observed at the high dose level. A significant increase

in uterine polyps (benign tumours) was found in mid and high dose females. The NOAEL was established at 750 mg/kg bw/day, the lowest dose studied.

In an additional study conducted in rats with 4000 mg/kg bw/day aspartame + DKP (3:1) no evidence for toxicity of aspartame with its degradation product DKP over 2 years in rats was seen (for further details see aspartame).

Reproductive and Developmental Toxicity

In rats, data from a male and female fertility study, an embryotoxicity study and a pre-postnatal development study with DKP administration in the diet are available. None of the endpoints studied showed a difference between treated groups and controls. The lowest NOAEL observed in these studies was 2000 mg DKP/kg bw/day, corresponding to the highest dose tested. In a further embryotoxicity study where a mixture of aspartame and DKP (3:1) was given in the diet, the NOAEL was also the highest dose studied of 3040 mg/kg bw/day.

Two developmental toxicity studies were performed in the rabbit which were confounded by poor health of animals and a number of deaths in treated groups possibly resulting from gavage problems. Maternal toxicity was observed in the first study at the high dose group (2000 mg/kg bw/day), therefore the NOAEL was established at 1000 mg/kg bw/day. In the second study, there was a statistical significant decrease in mean fetal weights at the highest dose; therefore the NOAEL was 1000 mg/kg bw/day.

Summary on DKP

DKP is one of the degradation products of aspartame formed under certain conditions. The content of DKP tested in the toxicological studies with aspartame varied from 0.1 up to 4%. Some toxicological studies were also performed with DKP itself. DKP is not absorbed from the gastrointestinal tract, but is transformed to phenyl acetic acid by gut bacteria and phenyl acetic acid is then absorbed. Overall, no genotoxic concern was identified for DKP. The lowest NOAEL observed from studies of chronic toxicity and carcinogenicity was 750 mg/kg bw/day. No carcinogenic effect was observed. Reproductive and developmental toxicity studies showed a lowest NOAEL in rats of 2000 mg DKP/kg bw/day and for rabbits of 1000 mg DKP/kg bw/day.

2.2. Toxicokinetics

A brief summary of the toxicokinetics of each, aspartame, its hydrolysis and degradation products is given in the EFSA report [12]. Exposure assessment is performed within the MoA analysis of EFSA mainly considering exposure in humans.

3. Clinical safety data

3.1. Safety in adult patients

There is no information available in the literature regarding the clinical safety profile of aspartame used in medications. Since 1998 the routine data mining signal detection of the WHO Global Individual Case Safety Report database Vigibase has reported rare cases of neurological adverse effects (convulsions, headache, and tremor) which can likely be due to the presence of aspartame in the medications.

3.2. Safety in special populations

PKU patients

Phenylalanine derived from aspartame is the major safety concern related with aspartame use for patients suffering from PKU. The safe use of aspartame at the ADI is not applicable to PKU patients. These individuals require total control of dietary phenylalanine intake to manage the risk from elevated phenylalanine levels. The intake of medicinal products containing aspartame should therefore also be avoided by PKU patients, respectively, warning statements informing about the aspartame content of the medicinal product must be included in the labelling.

Reference values for phenylalanine plasma levels were recommended by NIH for different PKU populations:

Children

The reference values for plasma L-phenylalanine concentration recommended by NIH [20] are L-phenylalanine levels between 120–360 µM for neonates through 12 years of age, and L-phenylalanine levels between 120–600 µM after 12 years of age.

Infants with PKU should start treatment to establish dietary control of their phenylalanine levels within 7–10 days of birth [20].

Pregnancy

According to NIH [20], L-phenylalanine levels between 120 and 360 µM should be achieved at least 3 months before conception and metabolic control should be achieved as soon as possible. Reference values for phenylalanine concentrations during pregnancy are between 120 and 360 µM.

4. Risk assessment

The basis for the ADI value of aspartame of 40 mg/kg bw/day previously set by SCF and JECFA was assumed to be derived from NOAELs of 4000 mg/kg bw/day identified in the chronic toxicity and carcinogenicity studies applying an uncertainty factor of 100.

4.1. Mode of action Approach applied by EFSA for the food additive aspartame

For the current risk assessment of aspartame, a mode of action (MoA) approach was applied by the EFSA panel. Re-analysing the whole data set, the EFSA panel focused on the effects reported in the rat and rabbit studies on reproductive and developmental toxicity. Aspartame is completely hydrolysed in the gastrointestinal tract to its metabolites phenylalanine, aspartic acid and methanol. Several studies in rat, dog, monkey and human reported that no aspartame could be detected in blood plasma after oral administration. Accordingly, aspartame itself cannot be responsible for reproductive and developmental toxicity.

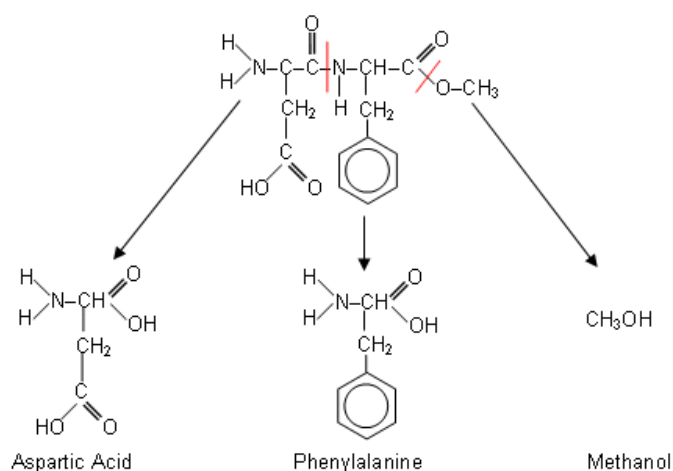


Fig. 3: Hydrolysis products of aspartame

Applying the Bradford Hill criteria and the decision tree of Boobis et al. 2008 [4], the human relevance of a MoA for toxicity observed in experimental animals was determined. The MoA proposed by the EFSA panel for aspartame was that the toxicological effects observed in rat and rabbit pregnancy were due to the metabolite phenylalanine.

Depression in pup body weight observed in high dose groups might be due to the high exposure to phenylalanine from metabolism of aspartame. This was supported by findings in rats and rabbits treated with phenylalanine which also showed a depression in pup body weight. A NOAEL of 2000 mg/kg bw/day was identified from the respective reproduction toxicity studies in rats. Rabbit studies were not considered for further evaluation because observed effects were attributed to the nutritional status as a result from gastrointestinal disturbances and were thus assumed to be species-specific in rabbits without direct relevance to humans.

Considering the biological plausibility of the MoA, there is established evidence for increased severity and frequency of adverse developmental effects with high phenylalanine plasma levels found in patients with PKU. Maternal PKU syndrome refers to the teratogenic effects of PKU during pregnancy. These effects include mental retardation, microcephaly, congenital heart disease, and intrauterine growth retardation. In untreated pregnancies wherein the mother has classic PKU with a plasma phenylalanine level greater than or equivalent to 1200 μM , abnormalities in offspring occur at exceedingly high frequencies, approximately 75–90% for microcephaly and mental retardation, and 15% for congenital heart disease. The form of the relation between prenatal phenylalanine exposure and offspring cognitive outcomes appears to be nonlinear, with no damage to the developing fetus until exposure passes a critical threshold level. The best estimate of the critical threshold of phenylalanine exposure without damage to the offspring occurs is 330 to 360 μM [29].

Malformations and mental retardation can be prevented by maintaining maternal phenylalanine plasma levels in a range of 120 to 360 μM through a phenylalanine restricted-diet.

Consideration was given to the fact whether effects observed on pup weight after aspartame exposure were caused by methanol released from aspartame. The lowest NOAEL for developmental and reproductive toxicity of methanol was derived from an inhalation study and was established at 560 mg/kg bw/day. On a weight basis the amount of methanol released from aspartame would be one tenth of the aspartame dose. Accordingly, a similar exposure to methanol from aspartame ingestion would occur at an oral aspartame dose of 5600 mg/kg bw/day. However, in studies on aspartame

effects were seen at doses of 1000 to 2000 mg/kg bw/day amounting to 100–200 mg/kg bw/day methanol which is below the NOAEL for reproductive and developmental toxicity of methanol.

No effects were observed in reproductive and developmental toxicity studies with L-aspartic acid when considering equivalent molar doses to the dose of aspartame (reproductive NOAEL of 1100 mg aspartic acid /kg bw/day would equal 2444 mg/kg bw/day aspartame). Therefore, it was concluded that effects observed following aspartame ingestion could not be due to aspartic acid.

Pharmacokinetic modelling

As a conclusion on the MoA analysis EFSA stated that the proposed MoA was relevant to humans based on the data from PKU patients. Therefore, for the risk characterisation, it was decided that the information on effects and dose response in PKU patients and human pharmacokinetic data were more appropriate than the results of animal studies of reproductive and developmental toxicity.

Accordingly, a dose-response modelling of plasma phenylalanine levels following aspartame administration was performed (for details refer to EFSA 2013 [12]). Pharmacokinetic data for a single oral bolus dose to represent the total daily aspartame intake were considered for modelling. This is a conservative approach since usually smaller intake levels throughout the day will be the rule. Furthermore, peak plasma levels at bolus were used as a surrogate for the response at the administered dose. A worst-case scenario also considered concomitantly dietary intake of phenylalanine. A maximum plasma concentration of 120 µM through dietary intake was assumed. Current clinical guidelines recommend plasma levels of phenylalanine below 360 µM. The concentration of plasma phenylalanine levels derived from aspartame was therefore set to 240 µM considering dietary intake (360–120 µM).

Based on the modelling, a plasma phenylalanine concentration of 240 µM would result from the administration of an oral bolus dose of 103 mg aspartame/kg bw (lower bound distributions: 88 mg aspartame/kg bw; 95th percentile, CI 59–125) to a normal subject. For a PKU heterozygous individual a bolus dose of 59 mg aspartame/kg would be necessary (lower bound distributions: 50 mg aspartame/kg bw; 95th percentile, CI 28–69).

Accordingly, for the normal and the PKU heterozygous population, a bolus administration of 40 mg/kg bw (which is equivalent to the current ADI) would not exceed the current clinical guideline of 360 µM. The EFSA panel concluded that the use of a sensitive subpopulation (PKU patients) obviated the need for a toxicodynamic uncertainty factor.

Summary of the EFSA opinion

Chronic toxicity and reproductive and developmental toxicity were the critical endpoints in the animal database. Based on a MoA analysis and the weight-of-evidence, the reproductive and developmental toxicity in animals was considered to be due to phenylalanine released from aspartame. The basis for the evaluation was therefore placed on human pharmacokinetic data of phenylalanine. Pharmacokinetic modelling showed that even in combination with the diet, a bolus administration of 40 mg/kg bw would not exceed the current clinical guideline of 360 µM for prevention of adverse effects on the fetuses of PKU mothers.

Therefore, there was no safety concern at the current ADI for aspartame. The ADI of 40 mg/kg bw/day was confirmed. ADI values established by EFSA, however, do not apply to infants below 12 weeks of age [10].

4.2. Risk assessment on the use of aspartame in medicinal products

For risk assessment on the use of aspartame in medicinal products it has to be considered, that EFSA's assessment is based on a worst-case scenario:

- A single bolus dose was used to represent the total daily intake, but usually small quantities will be consumed throughout the day.
- C_{max} levels were taken as surrogate for the administered dose not considering the short plasma half-life of phenylalanine of 1.7 hours in the healthy population [14].
- The critical phenylalanine plasma concentration to base comparisons was assumed to be clinically safe for pregnant women with PKU. The critical endpoint related to effects during pregnancy. This subgroup of the population will built up much higher phenylalanine plasma levels than normal or heterozygous subgroups. The true threshold for adverse effects from phenylalanine would be expected to be higher. For children born to PKU patients mild effects have been associated with mean phenylalanine plasma levels of 600–800 µM and significant detrimental effects have been associated with both mean and spike peak plasma levels of 1100–1200 µM during pregnancy.
- Studies on the effect of hourly ingestion of 600 mg aspartame over a period of eight hours by normal individuals have shown that plasma phenylalanine levels did not exceed the normal postprandial range [27]. This dose corresponded to approximately 80 mg aspartame/kg bw/day or twice the ADI. Using a published steady state model to compute the effect of repeated aspartame dosing on plasma phenylalanine levels the EFSA panel predicted that bolus administration of 40 mg/kg bw on an hourly basis was required to achieve steady state plasma levels greater than 240 µM.

Considering this worst case scenario of EFSA, the threshold doses calculated by EFSA and the resulting ADI will be neglected because they are irrelevant for people not suffering from PKU. People heterozygous with PKU do not show any symptoms and produce sufficient PAH from one allele to cope with normal phenylalanine use levels (EFSA 2013 [12], chapter 3.1.3.2). In one study, even after repeated dosing of up to 1800 mg aspartame/day to PKU heterozygous subjects, phenylalanine levels were within the normal postprandial levels (EFSA 2013 [12], chapter 3.1.3.3).

No changes will be made to the current information on the package leaflet i.e. only the information of the phenylalanine content of the medicinal product should be given for PKU patients. Otherwise no restrictions to the use of aspartame in medicinal products will be made. However, this ADI is not confirmed for children below the age of 12 weeks. Neither animal nor human data are available for this age group.

4.3. Risk Assessment of the impurity DKP

JECFA and SCF established an ADI for the degradation product DKP of 7.5 mg/kg bw/day [17, 22].

4.4 Risk assessment on the use of L-phenylalanine in medicinal products

L-Phenylalanine, the hydrolysis product of aspartame, is also used as an excipient. In combination with different other amino acids, L-phenylalanine is primarily added as stabiliser to infusions containing certain proteins.

For being an essential amino acid L-phenylalanine cannot be synthesised by the body and excessive restriction will have detrimental effects. L-Phenylalanine occurs naturally in foods, particularly protein-

rich food such as meat, fish and dairy products as constituent of proteins. The amount of L-phenylalanine introduced to the body by its use as excipient is far below the amount introduced by the diet. Therefore, no specific warning statements – apart from the statement concerning PKU patients – are needed for medicinal products that contain L-phenylalanine.

5. Safety information relevant for the package leaflet

Aspartame is fully hydrolysed in the gastrointestinal tract when orally ingested. The major hydrolysis products are L-aspartic acid, L-phenylalanine and methanol. Phenylalanine is further hydrolysed by phenylalanine hydroxylase (PAH) to tyrosine.

Mutations in the PAH gene result in phenylketonuria (PKU), an autosomal recessive inborn error of L-phenylalanine metabolism, causing hyperphenylalanaemia and low levels of tyrosine in the blood [27]. Untreated PKU is associated with an abnormal phenotype, including growth failure, microcephaly, seizures and intellectual impairment caused by accumulation of L-phenylalanine and its by-products. High phenylalanine levels are neurotoxic, mainly due to its inhibitory action on the transport of free L-amino acids necessary for protein and neurotransmitter synthesis.

Therefore, testing on PKU is performed within regular newborn screening. Management of PKU is primarily based on an L-phenylalanine restricted diet. Products containing aspartame or phenylalanine need to carry a respective warning statement being a source of phenylalanine. Such a warning statement is also included in the package leaflet of aspartame or phenylalanine containing medicinal products.

EFSA performed a re-assessment of the Acceptable Daily Intake (ADI) for aspartame in using a mode of action approach [12]. Since sufficient data on effects and dose response of L-phenylalanine were available from PKU patients, it was decided to base the risk characterisation rather on human than on animal data. As a result, EFSA was able to confirm the current ADI of 40 mg/kg/day.

EFSA's ADI calculation is based on data from PKU patients considering a worst case scenario of high aspartame intake and plasma levels. Therefore, the threshold doses calculated by EFSA and the resulting ADI are not directly applicable for use of medicinal products in people not suffering from PKU. Even people heterozygous with PKU do not show any symptoms and produce sufficient PAH from one allele to cope with normal phenylalanine use levels.

However, the amounts of aspartame reached by oral intake of medicinal products are generally well below the ADI. There are some medicinal products on the market with higher aspartame content but only for use in adults and children older than 12 years. The aspartame body burden of these products (on an mg/kg basis) is usually within the range of the ADI value and below the bolus dose of 103 mg/kg bw. This bolus dose of 103 mg/kg bw was calculated to result in an upper limit plasma phenylalanine concentration of 240 µM which was still considered as safe for PKU patients.

Accordingly, specific labelling requests for people not suffering from PKU are not applicable for aspartame in medicinal products.

No animal and clinical data are available for the use of aspartame in infants below the age of 12 weeks. This age group is also not included in EFSA's ADI calculation [9]. Therefore, a respective statement on the lack of data for infants below the age of 12 weeks and caution with use should be inserted in the package leaflet.

Abbreviations

ADI	Acceptable Daily Intake
ANS	Food Additives and Nutrient Sources Added to Food
Bw	body weight
CI	Confidence Interval
DKP	5-Benzyl-3,6-dioxo-2-piperazine-acetic acid
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organisation of the United Nations
GD	Gestation Day
IARC	International Agency for Research on Cancer
IP	intraperitoneal
IV	intravenous
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD	Lethal Dose
MoA	Mode of Action
NIH	US National Institutes of Health
NOAEC	No Observed Adverse Effect Concentration
NOAEL	No Observed Adverse Effect Level
PKU	Phenylketonuria
SCF	Scientific Committee on Food
WHO	World Health Organisation

References – Bibliography

1. Alexander, D.J., Collins, C.J., Coombs, D.W., Gilkison, I.S., Hardy, C.J., Healey, G., Karantabias, G., Johnson, N., Karlsson, A., Kilgour, J.D., McDonald, P., Association of Inhalation Toxicologists (AIT) Working Party, 'Recommendation for Standard Delivered Dose Calculation and Expression in Non-Clinical Aerosol Inhalation Toxicology Studies with Pharmaceuticals', *Inhalation Toxicology*, No. 20, 2008, p. 1179–1189.
2. Annex of the guideline 'Excipients in the labelling and package leaflet of medicinal products for human use' (EMA/CHMP/302620)
3. Apaja, M., 'Evaluation of toxicity and carcinogenicity of malonaldehyde: An experimental study in Swiss mice', *Acta Universitatis Ouluensis, Series D, Medica* 55, Finland: Anatomica, Pathologica, Microbiologica, No. 8, 1980, p. 61.
4. Boobis, A. R., Doe, J. E., Heinrich-Hirsch, B., Meek, M. E. B., Munn, S., Ruchirawat, M., Schlater, J., Seed, J., Vickers, C., 'IPCS framework for analyzing the relevance of a noncancer mode of action for humans', *Critical reviews in Toxicology*, No. 38, 2008, p. 87–96.
5. Brunner, R. L., Vorhees, C. V., Kinney, L., Butcher, R. E., 'Aspartame assessment of developmental psychotoxicity of a new artificial sweetener', *Neurobehavioural Toxicology* 1979; No 1, p. 79–86.
6. EFSA, 'Scientific Opinion of the Panel of Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) related to a new long-term carcinogenicity study on aspartame', *The EFSA Journal*, Vol. 356, 2006, p. 1–44.
7. EFSA, 'Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food on request from the European Commission related to the 2nd ERF carcinogenicity study on aspartame', *The EFSA Journal*, Vol. 945, 2009, p. 1–18.
8. EFSA, 'Updated Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food on request from the European Commission related to the 2nd ERF carcinogenicity study on aspartame taking into consideration study data submitted by the Ramazzini Foundation in February 2009', *The EFSA Journal*, Vol. 1015, 2009, p. 1–18.
9. EFSA, 'EFSA Statement on the scientific evaluation of two studies related to the safety of artificial sweeteners', *EFSA Journal*, Vol. 9(2), 2011, p. 2089.
10. EFSA, Scientific Opinion, 'Guidance for submission for food additive evaluations', *ANS, EFSA Journal*, Vol. 10(7), 2012, p. 2760.
11. EFSA, 'Guidance on default values to be used in the absence of measured data', *EFSA Journal*, Vol. 10(3), 2012, p. 2579.
12. EFSA, 'Scientific Opinion on the re-evaluation of aspartame (E951) as a food additive', *EFSA Journal*, Vol. 11(12), 2013, p. 3496.
13. European Pharmacopoeia 7.5.
14. Filer, L. J. and Stegink L. D., 'Aspartame metabolism in normal adults, phenylketonuric heterozygotes, and diabetic subjects, *Sugars and Sweeteners*', Eds. Kretchmer N. and Hollenbeck C.B., CRC Press, USA, 1991.
15. Guideline on excipients in the label and package leaflet of medicinal products for human use, CPMP/463/00 Rev. 1, July 2003.

16. Handbook of Pharmaceutical excipients, fifth Edition.
17. JECFA (Joint FAO/WHO Expert Committee on Food Additives), 'Toxicological evaluation of certain food additives: aspartame', WHO Food Additives Series No 15, World Health Organization, Geneva, 1980.
18. Lean, M.E.J., Hankey, C.R., 'Aspartame and its effects on health', British Medical Journal No. 329, 2004, p. 755–756.
19. Lord, G.H., Newberne, P.M., 'Renal mineralization – a ubiquitous lesion in chronic rat studies', Food and Chemical toxicology, 1990, No. 28, p. 449–455.
20. NIH (US National Institutes of Health), 'Phenylketonuria (PKU): screening and management', NIH Consensus Statement, 17(3), 2000, p. 1–27.
21. Ranney, R.E., Mares, S.E., Schroeder, R.E., Hutsell T.C., Radzialowski, F.M., 'The phenylalanine and tyrosine content of maternal and fetal body fluids from rabbits fed aspartame', Toxicology and Applied Pharmacology, 1975, Vol. 32, p. 339–346.
22. SCF (Scientific Committee on Food), 'Sweeteners', Reports of the Scientific Committee for Food (Twenty-first Series), EUR 11617 EN, Commission of the European Communities, Luxembourg, 1989.
23. Soffritti, M., Belpoggi, F., Cevolani, D., Guarino, M., Padovani, M. and Maltoni, C., 'Results of longterm experimental studies on the carcinogenicity of methyl alcohol and ethyl alcohol in rats', Annals of the New York Academy of Sciences, No. 982, 2002, p. 46–69.
24. Soffritti, M., Belpoggi, F., Degli Esposti, D., Lambertini, L., Tibaldi, E. and Rigano, A., 'First experimental demonstration of the multipotential carcinogenic effects of aspartame administered in the feed to Sprague-Dawley rats', Environmental Health Perspectives, No. 114, 2006, p. 379–385.
25. Soffritti, M., Belpoggi, F., Tibaldi, E., Degli Esposti, D. and Lauriola, M., 'Life-span exposure to low doses of aspartame beginning during pre-natal life increases cancer effects in rats', Environmental Health Perspectives, No. 115, 2007, p. 1293–1297.
26. Soffritti, M., Belpoggi, F., Manservigi, M., Tibaldi, E., Lauriola, M., Falcioni, L. and Bua, L., 'Aspartame administered in feed, beginning pre-natally through life span, induces cancers of the liver and lung in male Swiss mice', American Journal of Industrial Medicine, 2010, No. 53, p. 1197–1206.
27. Stegink, L. D., 'Aspartame metabolism in humans: acute dosing studies', Aspartame, Physiology and biochemistry, Eds. Stegink L. D. and Filer L. J. Jr. Marcel Dekker Inc., New York, USA, 1984, p. 509–553.
28. Stegink, L. D., Filer, L. J. Jr., Baker, G. L., 'Repeated ingestion of aspartame-sweetened beverage: effect on plasma amino acid concentrations in normal adults', Metabolism 38, 1988, p. 357–363.
29. Widaman, K. F. and Azen, C., 'Relation of Pre-natal Phenylalanine Exposure to Infant and Childhood Cognitive Outcomes: Results from the International Maternal PKU Collaborative Study', Pediatrics, No. 112, 2003, p. 1537–1543.

Annex 1 - Information in the package leaflet as per 2003 Guideline [15]

Name	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Aspartame (E951)	Oral	Zero	Contains a source of phenylalanine. May be harmful for people with phenylketonuria.	

Name	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Phenylalanine	All	Zero	This medicine contains phenylalanine. May be harmful for people with phenylketonuria.	