Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

VKM Report 2016:06

Risk assessment of "other substances" – L-phenylalanine and DL-phenylalanine

Opinion of the Panel on Nutrition, Dietetic Products, Novel Food and Allergy of the Norwegian Scientific Committee for Food Safety

Report from the Norwegian Scientific Committee for Food Safety (VKM) 2016: 06
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Assessed and approved

The opinion has been assessed by the Panel on Nutrition, Dietetic Products, Novel Food and Allergy of the Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM). Per Ole Iversen (chair), Livar Frøyland, Margaretha Haugen, Kristin Holvik, Martinus Løvik, Tor A Strand and Grethe S Tell.

(Panel members in alphabetical order after chair of the panel)

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Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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Summary

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has, at the request of the Norwegian Food Safety Authority (Mattilsynet; NFSA), assessed the risk of "other substances" in food supplements and energy drinks sold in Norway. VKM has assessed the risk of doses given by NFSA. These risk assessments will provide NFSA with the scientific basis while regulating the addition of "other substances" to food supplements.

"Other substances" are described in the food supplement directive 2002/46/EC as *substances other than vitamins or minerals that have a nutritional and/or physiological effect*. It is added mainly to food supplements, but also to energy drinks and other foods. VKM has not in this series of risk assessments of "other substances" evaluated any claimed beneficial effects from these substances, only possible adverse effects.

The present report is a risk assessment of L-phenylalanine and DL-phenylalanine and is based on previous risk assessments.

According to information from the Norwegian Food Safety Authority, L- and DL-phenylalanine are ingredients in food supplements sold in Norway. NFSA has requested a risk assessment of the following doses of L-phenylalanine and DL-phenylalanine in food supplements: L-phenylalanine 100, 250, 500, 750 and 1000 mg/day and DL-phenylalanine 50 and 75 mg/day.

L-phenylalanine is an essential amino acid which means it has to be obtained from the diet. Amino acids are building blocks for proteins and present in protein rich food such as milk, meat, fish, eggs and cheese.

No data on adverse health effects after chronic ingestion of supplemental phenylalanine in apparently healthy subjects are available, thus no tolerable upper intake level (UL) can be established. Patients with phenylketonuria (PKU), a genetic disorder that impairs phenylalanine hydroxylase (PAH), an enzyme involved in the metabolism of phenylalanine, must keep plasma levels of phenylalanine low in order to maintain normal growth and brain development. In Norway, all newborns are routinely screened for PKU three days after birth.

The mean dietary intake of phenylalanine in the EU population range from 0.4-4.1 g/day corresponding to 79.0 mg/kg bw per day for adolescents (10-17 years) and 58.7 mg/kg bw per day for adults, respectively (EFSA, 2013). The sweetener aspartame contains phenylalanine. Taking the molecular weight of phenylalanine into account, the proportion of to phenylalanine exposure from aspartame is 56%. The ADI of 40 mg aspartame/day/kg bw (providing 22.4 mg phenylalanine/day/kg bw) JECFA (1981) was re-evaluated and maintained in 2013, based on the notion that elevated plasma levels of phenylalanine in pregnant women leads to developmental toxicity in their children (EFSA, 2013).

The literature search did not provide novel information on adverse health effects related to intake of L-phenylalanine and no information related to DL-phenylalanine.

VKM concludes that:

- In adults (≥ 18 years), the specified doses 100, 250, 500, 750 and 1000 mg/day L-phenylalanine in food supplements are considered unlikely to cause adverse health effects.
- In adolescents (14 to < 18 years), the specified doses 100, 250, 500, 750 and 1000 mg/day L-phenylalanine in food supplements are considered unlikely to cause adverse health effects.
- In children (10 to < 14 years), the specified doses 100, 250, 500, 750 and 1000 mg/day L-phenylalanine in food supplements are considered unlikely to cause adverse health effects. Although the highest dose provides 23 mg/kg bw per day which slightly exceeds 22.4 mg/kg bw per day, it is considered unlikely to cause adverse health effects in healthy children 10 to < 14 years.
- None of the above conclusions are applicable for patients with phenylketonuria (PKU).
- No conclusion can be made regarding DL-phenylalanine.

Children below 10 years were not included in the terms of reference.

Short summary

At the request from the Norwegian Food Safety Authority (NFSA), the Norwegian Scientific Committee on Food Safety (VKM) has characterised the risk of specified doses of L-phenylalanine and DL-phenylalanine in food supplements. VKM concludes that:

- In adults (≥ 18 years), the specified doses 100, 250, 500, 750 and 1000 mg/day L-phenylalanine in food supplements are considered unlikely to cause adverse health effects.
- In adolescents (14 to < 18 years), the specified doses 100, 250, 500, 750 and 1000 mg/day L-phenylalanine in food supplements are considered unlikely to cause adverse health effects.
- In children (10 to < 14 years), the specified doses 100, 250, 500, 750 and 1000 mg/day L-phenylalanine in food supplements are considered unlikely to cause adverse health effects. Although the highest dose provides 23 mg/kg bw per day which slightly exceeds 22.4 mg/kg bw per day, it is considered unlikely to cause adverse health effects in healthy children 10 to < 14 years.
- None of the above conclusions are applicable for patients with phenylketonuria (PKU).
- No conclusion can be made regarding DL-phenylalanine.

Key words: Adverse health effect, phenylalanine, food supplement, negative health effect, Norwegian Scientific Committee for Food Safety, other substances, risk assessment, VKM.

Sammendrag på norsk

På oppdrag for Mattilsynet har Vitenskapskomiteen for mattrygghet (VKM) vurdert risiko ved tilsetting av «andre stoffer» i kosttilskudd og energidrikk som selges i Norge. VKM har risikovurdert ulike bruksdoser oppgitt fra Mattilsynet. Risikovurderingene gir et vitenskapelig grunnlag for Mattilsynet i arbeidet med å regulere bruken av «andre stoffer».

«Andre stoffer» er stoffer som har en ernæringsmessig eller fysiologisk effekt, og som ikke er vitaminer og mineraler. De tilsettes i hovedsak til kosttilskudd, men også til energidrikker og andre næringsmidler. I disse risikovurderingene har VKM ikke sett på påståtte gunstige helseeffekter, men kun vurdert mulige negative helseeffekter.

I denne rapporten har VKM vurdert risiko ved L-fenylalanin og DL-fenylalanin, og den er basert på tidligere risikovurderinger.

Ifølge informasjon fra Mattilsynet er L-fenylalanin og DL-fenylalanin ingredienser i kosttilskudd og energidrikker som selges i Norge. Oppdraget fra Mattilsynet var å risikovurdere følgende doser av L-fenylalanin og DL-fenylalanin i kosttilskudd: henholdsvis 100, 250, 500, 750 og 1000 mg/dag L-fenylalanin, og 50 og 75 mg/dag DL-fenylalanin.

L-fenylalanin er en essensiell aminosyre og vi må derfor få denne tilført via kosten vår. Aminosyrer inngår i proteiner og finnes i proteinrik mat slik som melk, kjøtt, fisk, egg og ost.

Man mangler tilstrekkelig kunnskap om mulige negative helseeffekter ved langvarig inntak av fenylalanin hos friske individer og det foreligger ingen øvre inntaksgrense. Pasienter med fenylketonuri (PKU; Føllings sykdom) tåler ikke fenylalanin og må følges nøye opp av helsepersonell slik at ikke mengden fenylalanin i blodet blir så høyt at det kan medføre redusert vekst og hjerneutvikling. Nyfødte blir rutinemessig kontrollert for PKU.

I EU er det gjennomsnittlige inntaket av fenylalanin i kosten 79 mg/kg kroppsvekt per dag for 10-17 åringer og 58,7 mg/kg kroppsvekt per dag for voksne (EFSA, 2013). Søtningsstoffet aspartam inneholder 56 prosent fenylalanin. En ADI på 40 mg aspartam/dag/kg kroppsvekt ble opprinnelig fastsatt av JECFA (1981). En reevaluering av ADI for aspartam ble gjennomført av EFSA og de opprettholdt en ADI på 40 mg aspartam/dag/kg kroppsvekt (noe som gir 22,4 mg L-fenylalanin/kg kroppsvekt per dag) med bakgrunn i at forhøyede nivåer av plasma fenylalanin hos gravide leder til redusert hjerneutvikling hos barna (EFSA, 2013).

Litteratursøket gav ikke ny informasjon knyttet til negative helseeffekter relatert til inntak av fenylalanin og det ble ikke funnet noe data vedrørende negative helseeffekter av DL-fenylalanin.

Vitenskapskomiteen for mattrygghet (VKM) konkluderer med at:

- For voksne (≥18 år) er det usannsynlig at dosene 100, 250, 500, 750 og 1000 mg/dag L-fenylalanin i kosttilskudd vil forårsake negative helseeffekter.
- For ungdom (14 til <18 år) er det usannsynlig at dosene 100, 250, 500, 750 og 1000 mg/dag L-fenylalanin i kosttilskudd vil forårsake negative helseeffekter.
- For barn (10 til <14 år) er det usannsynlig at dosene 100, 250, 500, 750 og 1000 mg/dag L-fenylalanin i kosttilskudd vil forårsake negative helseeffekter. Til tross for at den høyeste dosen tilsvarer 23 mg/kg kroppsvekt per dag, og dermed er en moderat overskridelse av "value for comparison" på 22,4 mg/kg kroppsvekt per dag, anses det som usannsynlig at dosen vil medføre negative helseeffekter hos friske barn/ungdom 10 til <14 år.
- Ingen av de ovennevnte konklusjonene gjelder for pasienter med fenylketonuri (Føllings sykdom).
- Det kan ikke gis noen konklusjoner for DL-fenylalanin.

Barn under 10 år inngår ikke i oppdraget.

Kort sammendrag

- For voksne (≥18 år) er det usannsynlig at dosene 100, 250, 500, 750 og 1000 mg/dag L-fenylalanin i kosttilskudd vil forårsake negative helseeffekter.
- For ungdom (14 til <18 år) er det usannsynlig at dosene 100, 250, 500, 750 og 1000 mg/dag L-fenylalanin i kosttilskudd vil forårsake negative helseeffekter.
- For barn (10 til <14 år) er det usannsynlig at dosene 100, 250, 500, 750 og 1000 mg/dag L-fenylalanin i kosttilskudd vil forårsake negative helseeffekter. Til tross for at den høyeste dosen tilsvarer 23 mg/kg kroppsvekt per dag, og dermed er en moderat overskridelse av "value for comparison" på 22,4 mg/kg kroppsvekt per dag, anses det som usannsynlig at dosen vil medføre negative helseeffekter hos friske barn/ungdom 10 til <14 år.
- Ingen av de ovennevnte konklusjonene gjelder for pasienter med fenylketonuri (Føllings sykdom).
- Det kan ikke gis noen konklusjoner for DL-fenylalanin.

Abbreviations and glossary

Abbreviations

ADI - acceptable daily intake
AESAN - the Scientific Committee of the Spanish Agency for Food Safety and Nutrition
bw - body weight
EFSA - European Food Safety Authority
IOM - Institute of Medicine, USA
JECFA - Joint FAO/WHO Expert Committee on Food Additives
NFSA - Norwegian Food Safety Authority [*Norw.*: Mattilsynet]
NIH - National Institutes of Health, USA
NOAEL - no observed adverse effect level
PAH - phenylalanine hydroxylase
PKU - phenylketonuria
UL - tolerable upper intake level
VKM - Norwegian Scientific Committee for Food Safety [*Norw.*: Vitenskapskomiteen for Mattrygghet]
WHO - World Health Organization

Glossary

"Other substances": a substance other than a vitamin or mineral that has a nutritional or physiological effect (European Regulation (EC) No. 1925/2006, Chapter I, Article 2; https://www.anses.fr/sites/default/files/documents/NUT2007sa0314EN.pdf).

"Negative health effect" and "adverse health effect" are broad terms and World Helath Organization (WHO) has established the following definition for "adverse effect": a change in morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences (WHO, 1994).

An adverse event is considered serious if it: results in death, is life-threatening, requires or prolongs hospitalization, is a congenital anomaly or birth defect, is a persistent or significant disability/incapacity, is another serious or important medical event.

Background as provided by the Norwegian Food Safety Authority

"Other substances" are substances other than vitamins and minerals, with a nutritional and/or physiological effect on the body. "Other substances" are mainly added to food supplements, but these may also be added to other foods and beverages, such as sports products and energy drinks. Ingestion of these substances in high amounts presents a potential risk for consumers.

In Norway, a former practice of classification of medicines had constituted an effective barrier against the sale of potentially harmful "other substances". Ever since this practice was changed in 2009, it has become challenging to regulate and supervise foods with added "other substances". Meanwhile, in the recent years, the Norwegian market has witnessed a marked growth in the sales of products containing "other substances". In 2011, food supplements containing "other substances" constituted more than 50% of the market share.

While within the European Economic Area, these substances fall under the scope of the European Regulation (EC) No. 1925/2006 on the addition of vitamins, minerals and certain other substances to foods and the European Regulation (EC) No 258/97 concerning novel foods and novel food ingredients, "other substances" remain largely unregulated. In order to ensure safe use of "other substances" many countries have regulated their use at a national level. For example, Denmark regulates these substances in a positive list i.e. a list of substances with maximal daily doses, permitted for use in food supplements and other foods (FVM, 2014).

NFSA is working on the establishment of a regulation on the addition of "other substances" to foods at a national level. The regulation will include a list of substances with permitted maximal doses, based on the substances and doses found in products on the Norwegian market. NFSA has therefore requested the Norwegian Scientific Committee for Food Safety (VKM) to assess the safety of "other substances" found on the Norwegian market. NFSA, in consultation with the industry, has compiled a list of "other substances" found in products marketed in Norway. Only substances with a purity of minimum 50% or concentrated 40 times or more have been included in the list. Substances regulated by other legislations like those for novel foods, food additives, aromas, foods for special medical purposes, etc. have been excluded from the list.

Terms of reference as provided by the Norwegian Food Safety Authority

The Norwegian Food Safety Authority (NFSA) requested the Norwegian Scientific Committee for Food Safety (VKM) to assess the safety of L-phenylalanine and DL-phenylalanine in food supplements at the following doses:
L-phenylalanine: 100, 250, 500, 750 and 1000 mg/day
DL-phenylalanine 50 and 75 mg/day

NFSA requested VKM to assess the safety of "other substances" (in accordance to the guidance document developed in Phase 2) at the doses specified (Phase 3).

Safety assessments for "other substances" present in food supplements shall be carried out for a general population, ages 10 years and above.

Assessment

1 Introduction

"Other substances" are described in the food supplement directive 2002/46/EC as *substances other than vitamins or minerals that have a nutritional or physiological effect*, and may be added to food supplements or e.g. energy drinks.

This risk assessment evaluates the substances L-phenylalanine and DL-phenylalanine *per se*, and no specific products. In this evaluation L-phenylalanine is often termed merely as phenylalanine.

VKM has in this series of risk assessments of "other substances" not evaluated any claimed beneficial effects from these substances, but merely possible adverse effects at specified doses used in Norway.

According to information from the Norwegian Food Safety Authority (NFSA), L-phenylalanine and DL-phenylalanine are ingredients in food supplements purchased in Norway and NFSA has requested a risk assessment of the following doses of L-phenylalanine and DL-phenylalanine in food supplements: L-phenylalanine: 100, 250, 500, 750 and 1000 mg/day DL-phenylalanine 50 and 75 mg/day.

L-phenylalanine is an essential amino acid and must therefore be supplied by the diet. The recommended aromatic amino acid requirement (phenylalanine or tyrosine) is set to 25 mg/kg bw per day (WHO, 2007). In the US the mean daily consumption of phenylalanine is estimated at 3.4 g/day (IOM, 2005) and in sedentary Europeans the mean daily intake was estimated to 3.8 g/day (AFSSA, 2007). The mean dietary intakes of phenylalanine in the EU population correspond to 79.0 mg/kg bw per day for adolescents (10-17 years) and 58.7 mg/kg bw per day for adults (EFSA, 2013).

2 Hazard identification and characterisation

2.1 Literature

In this risk assessment we have evaluated previous risk assessments of phenylalanine (L- and DL-phenylalanine) and articles retrieved from a literature search.

2.1.1 Previous risk assessments

Risks related to L-phenylalanine as an individual amino acid have previously been evaluated by (IOM, 2005), EFSA (2008), AESAN (2012) and as part of the food additive aspartame in an evaluation by EFSA (2013). DL-phenylalanine has been previously evaluated by JECFA and evaluated by EFSA (2008).

Dietary reference intakes, tolerable upper intake levels for individual amino acids, Institute of Medicine. USA, 2005

The IOM (2005) assessment stated that there are major differences in phenylalanine metabolism between humans and rodents and that adverse effects were not observed following acute single oral doses of L-phenylalanine up to 10 g in 13 adult men. Several human studies with the sweetener aspartame, which is 56 percent by weight phenylalanine, have used doses of aspartame providing 2 to 200 mg/kg bw per day L-phenylalanine with no apparent adverse effects.

However, the data were insufficient to establish a dose-response relationship and derive a tolerable upper intake level (UL) in apparently healthy subjects. Special considerations were made regarding phenylketonuria (PKU) and the importance of early detection and restriction of dietary phenylalanine in order to avoid irreversible brain damage, growth retardation, and dermatological abnormalities, particularly during infancy and early childhood.

Maternal hyperphenylalaninemia due to deficient phenylalanine hydroxylation is a recognized human teratogen (IOM, 2005). Because phenylalanine is actively transported across the placenta, a pregnant woman with PKU exposes her developing fetus to potentially harmful levels of phenylalanine. High maternal plasma phenylalanine levels are associated with high incidence of mental retardation, microcephaly, intrauterine growth delay, and congenital heart malformations in the fetus. Based on this they concluded that careful maintenance of low plasma phenylalanine levels in the mother through dietary control, before conception and throughout pregnancy may prevent the teratogenic effects of phenylalanine (IOM, 2005).

Opinion on Flavouring Group Evaluation 79, Consideration of amino acids and related substances evaluated by JECFA (63rd) meeting. EFSA, 2008

The EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food evaluated 19 amino acids including L-phenylalanine and DL-phenylalanine (EFSA, 2008) as flavouring substances. The EFSA panel noted that the intake of L-phenylalanine as a flavouring substance was not of safety concern at the estimated intake level as the human exposure through food is an order of magnitude higher than the anticipated levels of exposure as flavouring substance. For DL-phenylalanine the EFSA panel agreed with the JECFA conclusion that there was no safety concern at estimated levels of intake as flavouring substance. However, the estimated intake levels of L-phenylalanine and DL-phenylalanine as flavouring substances were 17 and 1.9 µg/capita/day, respectively.

Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on the use conditions for certain substances other than vitamins, minerals and plants in food supplements – 1. Spain, 2012

The Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) pointed out that a significant number of studies carried out prior to the 1980s concluded that increases in the intake of L-phenylalanine (diets enriched with 3-7% L-phenylalanine) implied an increase in the circulating levels of L-tyrosine (AESAN, 2012). Therefore, the toxic effects of L-phenylalanine were linked to those of L-tyrosine (Benevenga and Steele, 1984; Harper et al., 1970). Based on animal and human studies and the protein reference intake recommended by the WHO for the adult population (WHO, 2007), AESAN concluded that a maximum daily amount of 1900 mg for the sum of L-tyrosine and L-phenylalanine is acceptable from the safety point of view for use as a food supplement.

Opinion on the re-evaluation of aspartame (E 951) as a food additive. EFSA, 2013

The EU Commission asked the EFSA to re-evaluate the safety of food additives already permitted in the EU before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedures and deadlines that are enshrined in the Regulation (EU) No 257/2010 of 25 March 2010 setting up a program for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

This evaluation points out that aspartame is rapidly and completely hydrolysed in the gastrointestinal tract to phenylalanine, aspartic acid and methanol. Phenylalanine at high plasma levels is known to cause developmental toxicity in humans. The EFSA Panel on Food Additives and Nutrient Sources added to Food concluded that human data on developmental toxicity were more appropriate for the risk assessment. Concentration-response modelling was used to determine the effects of aspartame administration on plasma phenylalanine using human data after phenylalanine administration to normal, PKU heterozygote or PKU homozygote individuals.

In healthy and PKU heterozygotes, aspartame intakes up to the ADI of 40 mg/kg bw per day (equivalent to 22.4 mg/kg bw per day phenylalanine) in addition to dietary phenylalanine, would not lead to peak plasma phenylalanine concentrations above the current clinical guideline (360 μM) for the prevention of adverse effects in fetuses (EFSA, 2013).

This ADI is not applicable to PKU patients.

2.1.2 Literature search

Literature searches were performed in MEDLINE and EMBASE in order to retrieve publications on adverse effects caused by phenylalanine. These databases were chosen to ensure comprehensive study retrieval. The literature search was performed 6 July 2015. Both human and animal studies were included in the search, and the search was limited back in time to 2005 when the report on tolerable upper levels for amino acids was published (IOM, 2005). The strategy for the search is included in Appendix 1.

2.1.2.1 Publication selection and data extraction

The literature search identified 384 articles. In the primary screening titles and abstracts of all unique publications retrieved were independently screened against the inclusion criteria checklist.

Inclusion criteria checklist:

- An adverse effect/adverse effects in relation to the substance alone is addressed
- Route of exposure for humans = oral
- Route of exposure for animals = oral, in addition, subcutaneous exposure is included if the toxicokinetic is equal as by oral exposure
- Human studies are performed in apparently healthy individuals or patient groups assumed to have normal phenylalanine absorption and metabolism.
- Animal model studies address adverse effects relevant to human health

The inclusion criteria checklist was developed by members of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics and the Panel on Nutrition, Dietetic Products, Novel Food and Allergy. Articles that did not appear to meet the inclusion criteria were excluded from further analysis. In situations where it was unclear whether the publication was of relevance to the risk assessment, it was retained for further screening. The primary screening was performed independently by two persons.

The full text of articles that passed the primary screening was retrieved for secondary screening. In this screening, the full text articles were reviewed and compared against the inclusion criteria checklist. The secondary screening was performed by the author of this opinion and in the secondary screening none of the articles fulfilled the inclusion criteria and they were excluded. However, one risk-assessment on aspartame (EFSA, 2013) was identified in a manual search and included in the results in this report (see Figure 2.1.2.1-1).

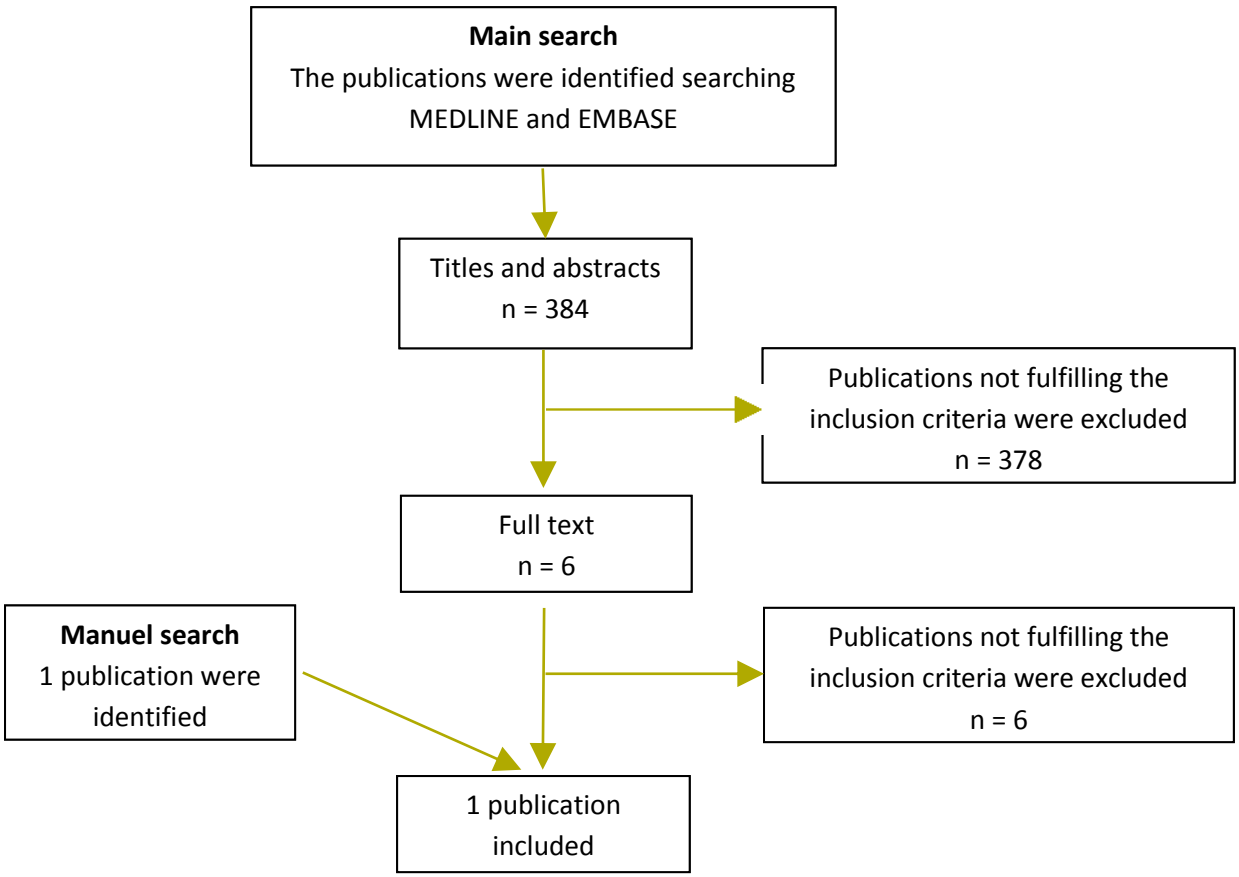

Figure 2.1.2.1-1: Flowchart for publication selection for phenylalanine literature search.

2.2 General information

There are two forms of phenylalanine: L-phenylalanine and the synthetic stereoisomer D-phenylalanine. DL is a mixture of D- and L-phenylalanine resulting from bacterial or chemical synthesis of the amino acid. The mix is termed DL-phenylalanine.

2.2.1 Chemistry

L-phenylalanine (2-amino-3-phenylpropanoic acid) is an essential α-amino acid with a benzene ring and the chemical formula $C_9H_{11}NO_2$. The CAS number is 63-91-2 and the EFSA flavouring number is 17.018.

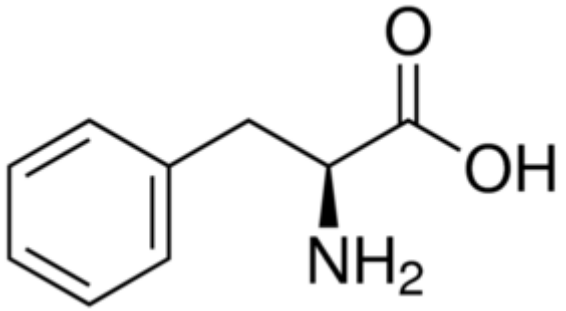

Figure 2.2.1-1: Structural formula for L-phenylalanine.

D-phenylalanine is the stereoisomer of L-phenylalanine, but the biological functions remain unclear. The CAS number is 673-06-3.

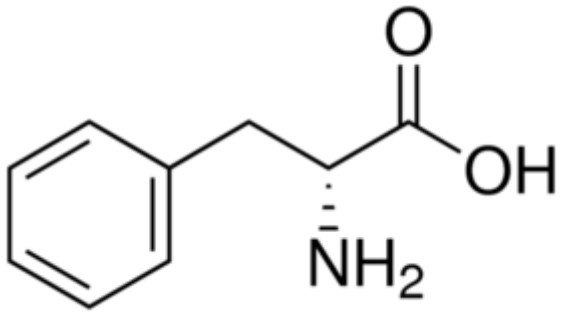

Figure 2.2.1-2: Structural formula for D-phenylalanine.

DL-phenylalanine is a mixture of L-phenylalanine and D-phenylalanine. The CAS number is 150-30-1 and the EFSA flavouring number is 17.017.

2.2.1 Occurrence

In the normal diet, the amino acids are ingested as components of food proteins and not as free amino acids. L-phenylalanine is present in protein rich food such as milk, meat, fish, eggs and cheese. In addition, L- and DL-phenylalanine are available in food supplements. L-phenylalanine is utilised in the manufacture of food and drink products and is sold as a food supplement. Additionally, L-phenylalanine is part of the sweetener aspartame, a dipeptide of L-phenylalanine methyl ester and L-aspartic acid.

In contrast to L-phenylalanine and DL-phenylalanine, D-phenylalanine is not an approved flavouring substance in EU. DL-phenylalanine is sold as a food supplement.

2.3 Absorption, distribution, metabolism and excretion

2.3.1 In humans

L-phenylalanine is an essential amino acid required for protein synthesis and has both glycogenic and ketogenic properties. It is the precursor of the amino acid tyrosine.

Free α-amino acids, whether ingested as such in the form of food supplements or released after the digestion of proteins by proteolytic enzymes, are absorbed as free amino acids or in small peptides primarily through the intestinal mucosa and enter the portal blood. Once absorbed, a variety of carrier systems transport α-amino acids into cells (Kilberg, 1982). These amino-acid carriers are mostly sodium ion-dependent systems that are specific to a particular class of α-amino acids (e.g. neutral amino acids with short side-chains, neutral amino acids with branched or aromatic side-chains, basic amino acids, and dicarboxylic amino acids). The carrier systems are adaptive and under hormonal regulatory control. Although small amounts of di-, tri-, and polypeptides may be absorbed by a transport system involving membrane-bound γ-glutamyl transferase, most amino acids enter the cells unchanged (Nelson and Cox, 2000). After absorption, α-amino acids are used in protein synthesis or rapidly metabolised to intermediates in the citric acid cycle, as evidenced by the presence of only trace amounts of α-amino acids in the plasma. The excretion of α-amino acids is regulated by renal tubular reabsorption, in which the proximal tubules conserve α-amino acids. The daily excretion of α-amino acids in the urine amounts to only 20–150 mg/day in humans (Tietz, 1986). Minimal loss of α-amino acids occurs in the urine and faeces.

The extent of racemisation of L-amino acid residues to D-isomers in food proteins increases with pH, time, and temperature. The nutritional utilisation of different D-amino acids varies widely, both in animals and humans. In addition, some D-amino acids may be deleterious. The antimetabolic effect of D-tyrosine can be minimised by increasing the L-phenylalanine content of the diet (Friedman, 1991).

Under normal conditions, α-amino acids that are not required for de novo protein synthesis undergo catabolism primarily in the liver, with the exception of the branched amino acids, which undergo degradation in muscle, adipose, kidney and brain tissues. There is no storage of amino acids in humans. The amino acids undergo a process called oxidative deamination in which most amino acids are transformed to α-ketoacids and NH_4^+. The α-ketoacids are completely oxidised to CO_2 and water or provide three or four carbon units that are converted via gluconeogenesis to yield glucose, or via ketogenesis to yield ketone bodies (Nelson and Cox, 2000). The amino groups resulting from transamination of most of the amino acids are transferred by transaminases to α-ketoglutarate to form L-glutamate in the cytosol of hepatocytes. L-glutamate then undergoes deamination in the mitochondria yielding NH_4^+ and α-ketoglutarate via L-glutamate dehydrogenase. The ammonium ion is either used in other metabolic pathways or converted in the liver to urea for excretion via the kidneys.

Phenylalanine hydroxylase (PAH) catalyses the stereospecific hydroxylation of L-phenylalanine to tyrosine whereas a phenylalanine transaminase yields phenylpyruvate (Figure 2.3.1.).

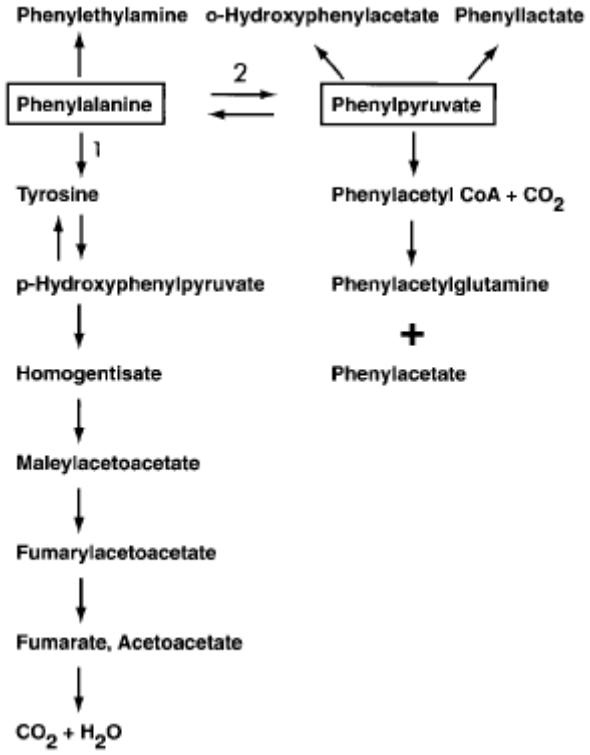

Fig. 2.3.1-1: Phenylalanine metabolism. 1) Phenylalanine hydroxylase and 2) phenylalanine transaminase (from Kaufman (1999)).

PKU is an autosomal recessive disease characterised by a lack of hepatic phenylalanine hydroxylase activity. Untreated PKU may result in growth failure, seizures and intellectual impairment caused by the accumulation of L-phenylalanine and its byproducts (for an overview see EFSA (2013). Blood phenylalanine levels above 1200 µM, i.e. 20 times above normal levels (50-60 µM), is associated with PKU. If not treated by a low L-phenylalanine diet from early infancy it will lead to postnatal brain damage and mental retardation (Scriver et al., 1989). Mutations in the PAH gene may vary and heterozygotes have a slower, but adequate metabolism and clearance (Stegink et al., 1980). In Norway PKU heterozygotes are considered healthy (Rina Lilje, at the PKU unit at Oslo University Hospital-Rikshospitalet, Norway, personal communication).

No studies on DL-phenylalanine evaluating adverse effects were identified.

2.3.2 Animal studies

IOM (2005) points out that because of major species differences in phenylalanine metabolism between humans and rodents (Clarke and Bier, 1982; Moldawer et al., 1983),

studies in which high doses of L-phenylalanine were fed to rodents could not be utilised in developing a UL for L-phenylalanine. There is one study indicating that high concentrations of L-phenylalanine (3 g/kg bw per day) fed to monkeys from a few days after birth until 2 or 3 years of age can produce irreversible brain damage (Waisman and Harlow, 1965). However, this study did not provide any dose–response data.

JECFA (2006) discussed a limited number of toxicity studies in young Wistar rats given water containing 7% L-phenylalanine by gavage from postnatal days 1 to 21. At day 21, they were placed on a solid diet supplemented with 7% L-phenylalanine for 7 days. This dietary level corresponds to an average daily intake of L-phenylalanine of approximately 7000 mg/kg bw. There were no concurrent controls in this study. A high rate of mortality was reported and the rats demonstrated signs of toxicity which included lesions of the eyes, swollen toes and some toe atrophy, as well as difficulty in urinating. Necropsy revealed swelling of the bladder and obstruction of the urethra in the more severe cases (number unspecified). The urine contained small white crystals, which were identified as primarily tyrosine, a metabolite of phenylalanine (Dolan and Godin, 1966).

2.4 Toxicological data/Adverse effects

2.4.1 Human studies

In 2005 IOM concluded that data were not available on the effects of chronic ingestion of supplemental phenylalanine by apparently healthy adults. Adverse effects were not evident following acute single oral doses of L-phenylalanine as high as 10 g in 13 adult men (Ryan-Harshman et al., 1987).

Most of the literature on the consumption of large doses of L–phenylalanine consists of studies on the effects of large doses of the artificial sweetener aspartame, which is 56 percent by weight L-phenylalanine. In adults given oral doses of aspartame ranging from 4 to 200 mg/kg bw per day (2 to 100 mg/kg bw per day of L-phenylalanine), dose-related increases in plasma phenylalanine were observed (Filer and Stegink, 1988). Ingestion of single doses up to 60 mg/kg bw per day aspartame (30 mg/kg bw per day of L-phenylalanine) by normal weight adults had no effect on behavior or cognitive performance (Lieberman et al., 1988; Stokes et al., 1991). The data on the adverse effects of L-phenylalanine intake from supplements were not available for a dose-response assessment and derivation of a UL in apparently healthy humans (IOM, 2005). No information was provided regarding DL-phenylalanine.

Mutations in the PAH gene may vary and heterozygotes have a slower (approximately 50 percent), but adequate metabolism and clearance (Bremer and Neumann, 1966; Stegink et al., 1980; Woolf et al., 1967). A temporary plasma concentration above 360 μM (6 mg per dL) is unlikely to cause adverse effects in healthy and PKU heterozygote individuals. The EFSA (2013) report on aspartame concluded that in normal and PKU heterozygotes, aspartame intakes up to the ADI of 40 mg/kg bw per day (i.e. 22.4 mg phenylalanine/kg bw

per day), in addition to dietary phenylalanine, would not lead to peak plasma phenylalanine concentrations above the current clinical guideline (360 μM/6 mg per dL) for the prevention of adverse effects in fetuses.

2.4.1.1 Interactions

No information concerning interactions between phenylalanine and other nutrients, drugs or any other substance were identified in the studies reviewed in this risk assessment. The absence of information in the selected literature does not document an absence of interactions.

2.4.1.2 Allergic sensitisation (including adjuvant effects)

There was no information concerning allergic sensitisation or allergy adjuvant effects in the literature reviewed in the present risk assessment. The absence of information in the selected literature does not document an absence of allergic sensitisation or allergy adjuvant effects. Mode of action for adverse effects

2.4.2 Vulnerable groups

As described in IOM (2005) PKU is a genetic disorder that impairs phenylalanine hydroxylase (PAH) activity. Impaired PAH activity allows phenylalanine or its catabolic byproducts to accumulate above normal levels in the plasma during critical periods of brain development. Persistently elevated levels of L-phenylalanine in the plasma before and during infancy and childhood can result in irreversible brain damage, growth retardation, and dermatologic abnormalities if dietary phenylalanine is not restricted within one month after birth and continued at least through childhood and adolescence (Scriver et al., 1989).

Restriction of phenylalanine intake throughout life in PKU patients is necessary to keep plasma phenylalanine levels low and to promote normal growth and brain development (Scriver et al., 1989). A pregnant woman with PKU exposes her developing fetus to potentially harmful levels of phenylalanine. High maternal plasma phenylalanine levels are associated with high incidence of mental retardation, microcephaly, intrauterine growth delay, and congenital heart malformations in the fetus (Scriver et al., 1989). Careful maintenance of plasma phenylalanine levels in the mother through dietary control, before conception and throughout her pregnancy, may prevent the teratogenic effects of phenylalanine.

PKU diets consist mainly of low-protein natural foods (vegetables, fruits and some cereals) that are modest in L-phenylalanine (Giovannini et al., 2012). The aim of the dietary treatment is to avoid acute and chronic increased concentrations of L-phenylalanine in plasma and consequently in cerebral tissue, responsible for an important worsening of the neuronal performance and behaviour in PKU patients. High levels of plasma L-phenylalanine during infancy and childhood lead to severe, permanent brain damage, but the exact

mechanism of the effects on neuronal function is not understood. According to the US National Institutes of Health (NIH) Consensus Statement on phenylketonuria, it is assumed that levels in excess of dietary requirement for this essential amino acid but below 10 mg/dL (600 µM) do not lead to brain damage (NIH, 2000).

The reference values for plasma L-phenylalanine concentration recommended by NIH (2000) are L-phenylalanine levels between 120-360 µM (2–6 mg/dL) during pregnancy and for neonates through 12 years of age, and L-phenylalanine levels between 120-600 µM (2–10 mg/dL) after 12 years of age. EFSA (2013) recommended that L-phenylalanine levels between 120 and 360 µM are achieved at least 3 months before conception and that metabolic control should be achieved as soon as possible.

2.5 Summary of hazard identification and characterisation

No data on adverse health effects after chronic ingestion of supplemental phenylalanine in apparently healthy subjects are available and no UL could be established. However, patients with PKU, a genetic disorder that impairs phenylalanine hydroxylase (PAH) must keep plasma levels of phenylalanine low in order to maintain normal growth and brain development.

In the recent re-evaluation of aspartame as a food additive it was concluded that the ADI of 40 mg/kg bw per day was maintained and it was based on that elevated plasma levels of phenylalanine in pregnant women leads to developmental toxicity in their children. The sweetener aspartame contains phenylalanine. Taking the molecular weight of phenylalanine into account, the factor for aspartame exposure to phenylalanine exposure is 56%. The ADI of 40 mg aspartame/kg bw per day provides 22.4 mg phenylalanine/kg bw per day representing the value of comparison in this risk assessment. Although an ADI is used for food additives and not for supplements, the lack of an UL and the fact that the ADI of aspartame was set due to developmental toxicity from elevated plasma phenylalanine exposure in pregnant women VKM will use 22.4 mg/kg bw per day as value for comparison in the risk characterisation of L-phenylalanine.

No value for comparison for DL-phenylalanine could be set.

3 Exposure / Intake

Exposure of L-phenylalanine and DL-phenylalanine was estimated from the intake of food supplements. For food supplements, the intake of L-phenylalanine and DL-phenylalanine was estimated for the age groups 10-14 years, 14-18 years and adults (≥18 years).

3.1 Food supplements

The Norwegian Food Safety Authority has requested a risk assessment of 100, 250, 500, 750 and 1000 mg/day L-phenylalanine and 50 and 75 mg/day DL-phenylalanine in food supplements for children 10 years and above, adolescents and adults. The default body weights (bw) for age groups determined by the EFSA were used: 10 to <14 years=43.4 kg, 14 to <18 years=61.3 kg, and adults=70 kg. The intakes per kg bw are given in Tables 3.1-1 and 3.1-2).

Table 3.1-1: Estimated exposure of L-phenylalanine from specified doses in food supplements in children, adolescents and adults.

Groups	Daily doses, mg	Body weight	Exposures (mg/kg bw per day)
Children (10 to <14years)	100, 250, 500, 750 and 1000	43.4	2, 6, 12, 17 and 23
Adolescent (14 to <18 years)	100, 250, 500, 750 and 1000	61.3	2, 4, 8, 12 and 16
Adults (≥18 years)	100, 250, 500, 750 and 1000	70.0	1, 4, 7, 11 and 14

Table 3.1-1: Estimated exposure of DL-phenylalanine from specified doses in food supplements in children, adolescents and adults.

Groups	Daily doses, mg	Body weight	Exposures (mg/kg bw per day)
Children (10 to <14years)	50 and 75	43.4	1 and 2
Adolescent (14 to <18 years)	50 and 75	61.3	0.8 and 1
Adults (≥18 years)	50 and 75	70.0	0.7 and 1

3.2 Other sources

Dietary sources of phenylalanine are foods rich in protein (meat, fish, eggs, bread, dairy products, nuts, and seeds) and foods and drinks containing aspartame, flour, soya, beer or cream liqueurs.

The mean dietary intakes of phenylalanine in the EU population range from 0.9 g/day (corresponding to 93.0 mg/kg bw per day for toddlers) up to 4.1 g/day (corresponding to 58.7 mg/kg bw per day for adults). In adolescents (10-17 years) the intake of dietary phenylalanine corresponded to 79 mg/kg bw per day (EFSA, 2013). An additional intake of up to 22.4 mg L-phenylalanine/kg bw per day from the sweetener aspartame is unlikely to cause adverse health effects (EFSA, 2013).

4 Risk characterisation

The specified doses received from NFSA are L-phenylalanine: 100, 250, 500, 750 and 1000 mg/day and DL-phenylalanine: 50 and 75 mg/day in food supplements, and the exposure for adults, adolescents and children at or above 10 years are given in chapter 3.

The value for comparison used in this risk characterisation of L-phenylalanine is 22.4 mg/kg bw per day. This is based on the EFSA (2013) opinion for aspartame.

This value is not applicable for patients with PKU.

The literature search did not provide information on adverse health effects related to intake of L-phenylalanine and no information related to DL-phenylalanine.

No risk characterisation can be made for DL-phenylalanine.

No studies with L-phenylalanine in children were found. However, there are no data indicating that children and adolescent are more vulnerable than adults for phenylalanine. No tolerance level is set for phenylalanine specifically for children or adolescents. Assuming similar tolerance for these age groups as for adults, the same value for comparison as for adults is used for children and adolescents.

VKM considers that:

In adults (≥ 18 years), the specified doses 100, 250, 500, 750 and 1000 mg/day L-phenylalanine in food supplements are considered unlikely to cause adverse health effects.

In adolescents (14 to < 18 years), the specified doses 100, 250, 500, 750 and 1000 mg/day L-phenylalanine in food supplements are considered unlikely to cause adverse health effects.

In children (10 to < 14 years), the specified doses 100, 250, 500, 750 and 1000 mg/day L-phenylalanine in food supplements are considered unlikely to cause adverse health effects. Although the highest dose provides 23 mg/kg bw per day which slightly exceeds 22.4 mg/kg bw per day, it is considered unlikely to cause adverse health effects in healthy children 10 to < 14 years.

None of these considerations are applicable for patients with PKU.

5 Uncertainties

The literature search was not described in the IOM (2005), EFSA (2008), AESAN (2012)and EFSA (2013) making it difficult to assess how well the literature is covered. However, the latter report has a thorough description of the models including various uncertainty analyses. None of the included studies provide novel data regarding L-phenylalanine compared to the previous risk assessments and no studies addressing adverse health effects from DL-phenylalanine were identified. The literature search in the present risk characterisation went back to 2005, and limited to scientific studies in English or Scandinavian languages identifying a search term in the abstract. Thus, relevant studies could have been excluded unintentionally. There is also a possibility that our search strategy failed as some RCTs may have reported adverse effects but not in the abstract. The importance of reporting adverse effects in the abstract has been made clear by the "Consort group" (http://www.consort-statement.org).

6 Conclusions with answers to the terms of reference

The Norwegian Food Safety Authority (NFSA) requested the Norwegian Scientific Committee for Food Safety (VKM) to assess the safety of L-phenylalanine and DL-phenylalanine in food supplements at the doses 100 to 1000 mg/day and 50 to 75 mg/day, respectively for the general population above 10 years of age.

The literature search did not provide novel information on adverse health effects related to intake of L-phenylalanine for the general population (above 10 years of age) and no information related to DL-phenylalanine.

Based on previous risk assessments values for comparisons used in this risk characterisation of L-phenylalanine, VKM will use 22.4mg/kg bw per day for all age groups. No value for comparison for DL-phenylalanine could be set.

VKM concludes that:

- In adults (≥ 18 years), the specified doses 100, 250, 500, 750 and 1000 mg/day L-phenylalanine in food supplements are considered unlikely to cause adverse health effects.
- In adolescents (14 to < 18 years), the specified doses 100, 250, 500, 750 and 1000 mg/day L-phenylalanine in food supplements are considered unlikely to cause adverse health effects.
- In children (10 to < 14 years), the specified doses 100, 250, 500, 750 and 1000 mg/day L-phenylalanine in food supplements are considered unlikely to cause adverse health effects Although the highest dose provides 23 mg/kg bw per day which slightly exceeds 22.4 mg/kg bw per day, it is considered unlikely to cause adverse health effects in healthy children 10 to < 14 years.
- None of the above conclusions are applicable for patients with phenylketonuria (PKU).

No conclusion can be made regarding DL-phenylalanine.

An overview of the conclusions is presented in Table 6.1.

Table 6.1: An overview of the conclusions for DL-phenylalanine and L-phenylalanine in food supplements.
Green: Estimated exposures to L-phenylalanine are unlikely to cause adverse health effects.
Grey: No conclusion can be made.

	DL-phenylalanine		**L-phenylalanine**				
Doses / Age groups	50 mg/day	75 mg/day	100 mg/day	250 mg/day	500 mg/day	750 mg/day	1000 mg/day
Children (10 to <14 years)	No Conclusion	No Conclusion					
Adolescents (14 to <18 years)	No Conclusion	No Conclusion					
Adults (≥18 years)	No Conclusion	No Conclusion					

7 Data gaps

There is a general lack of studies of adverse effects as primary outcomes of supplemental L-phenylalanine and particularly DL-phenylalanine in the general population, both for sub-chronic and chronic toxicity studies.

There are especially few studies on adverse health effects related to DL-phenylalanine in general.

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9 Appendix

Search strategy for this risk assessment

Database: Ovid MEDLINE(R) <1946 to June Week 4 2015>, Embase <1974 to 2015 July 06>

1. phenylalanine*.ti. (13684)
2. (risk* or safety or adverse or side-effect*1 or hazard* or harm* or negative or contraindicat* or contra-indicat* or interact* or toxicity or toxic).tw. (8840135)
3. 1 and 2 (1924)
4. (conference abstract* or letter* or editorial*).pt. (4498567)
5. 3 not 4 (1860)
6. limit 5 to (danish or english or norwegian or swedish) (1774)
7. remove duplicates from 6 (999)
8. limit 7 to yr="2005 -Current" (384)