Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

VKM Report 2017:11

Risk assessment of "other substances" – L-aspartic acid

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) 2017: 11
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Risk assessment of "other substances" – L-aspartic acid

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Assessed and approved

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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 in food supplements and concentrations in energy drinks given by NFSA. These risk assessments will provide NFSA with the scientific basis while regulating the addition of "other substances" to food supplements and other foods.

"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 specified doses of L-aspartic acid in food supplements, and it is based on previous risk assessments and articles retrieved from literature searches.

According to information from NFSA, L-aspartic acid is an ingredient in food supplements sold in Norway. NFSA has requested a risk assessment of 3000, 3500, 4000, 4500, 5000 and 5700 mg/day of L-aspartic acid in food supplements.

L-aspartic acid is a dispensable dicarboxylic amino acid that can be produced by the transamination of oxaloacetic acid, an intermediate in the metabolism of e.g. glucose and some amino acids. L-aspartic acid is present in frequently consumed foods of animal and plant origin and is also a component of the sweetener aspartame. Dietary intake of aspartic acid in Norway is not known, but data from NHANES III (USA) suggest a mean dietary intake of about 6.5 g/day in adults. The highest intake was seen in men 31 through 50 years of age at the 99th percentile of 15.4 g/day.

In the literature review we did not identify any long-term studies in human individuals that could be used for risk assessment. Short-term human studies found no adverse health effect when L-aspartic acid was given in acute doses ranging from 1 to 10 g/day, for time periods between one single dose and four weeks. None of these studies were undertaken to assess the toxicity of L-aspartic acid.

In the literature search, two animal studies were identified of which one was a 90-day subchronic toxicity study. In that study, a no observed adverse effect level (NOAEL) of 697 mg/kg bw per day in male rats and 715 mg/kg bw per day in female rats was established. No neurotoxicity was found, however a toxic effect on kidneys and possibly salivary glands was observed at 1400 mg/kg bw per day (lowest observed adverse effect level, LOAEL). For the risk characterisation, the NOAEL of 697 mg/kg bw per day derived

from the abovementioned subchronic toxicity study in rats was used for comparison with the estimated exposures from food supplements. The calculated Margin of Exposure (MOE) values for this NOAEL ranged from 5 to 16 for a daily intake of 3000-5700 mg/day of L-aspartic acid. These low MOE-values may not be regarded as acceptable since L-aspartic acid has caused toxic effects on the kidneys (regenerative renal tubules with tubular dilation) and acinar cell hypertrophy of salivary glands in rats. Further, direct information regarding potential adverse health effects in humans is not available due to absence of long-term studies.

In adults (≥18 years), adolescents (14 to < 18 years) and children (10 to < 14 years), the specified doses 3000, 3500, 4000, 4500, 5000 and 5700 mg/day L-aspartic acid in food supplements may represent a risk of adverse health effects.

Children younger than 10 years were not within the scope of the present risk assessment.

Short summary

At the request of the Norwegian Food Safety Authority, the Norwegian Scientific Committee for Food Safety (VKM) has characterised the risk of specified doses of L-aspartic acid in food supplements. VKM concludes that:

In adults (≥18 years), adolescents (14 to < 18 years) and children (10 to < 14 years), the specified doses 3000, 3500, 4000, 4500, 5000 and 5700 mg/day L-aspartic acid in food supplements may represent a risk of adverse health effects.

Key words: L-aspartic acid, aspartate, food supplement, adverse health effect, negative health effect, Norwegian Food Safety Authority, 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 doser brukt av kosttilskudd og konsentrasjoner i energidrikker oppgitt fra Mattilsynet. Disse risikovurderingene vil gi Mattilsynet vitenskapelig grunnlag for å regulere andre stoffer.

«Andre stoffer» er beskrevet i kosttilskuddsdirektivet 2002/46/EC som *stoffer som har en ernæringsmessig og/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.

Denne rapporten er en risikovurdering av L-aspartat, og den er basert på tidligere risikovurderinger og artikler hentet fra litteratursøk.

I følge informasjon fra Mattilsynet er L-aspartat en ingrediens i kosttilskudd som selges i Norge. Oppdraget fra Mattilsynet var å risikovurdere inntak på 3000, 3500, 4000, 4500, 5000 and 5700 mg/dag L-aspartat i kosttilskudd.

L-aspartat er en ikke-essensiell aminosyre med to karboksylgrupper. Den dannes ved transaminering av oksaleddiksyre, en metabolitt i omsetningen av bl.a. glukose og enkelte aminosyrer. L-aspartat finnes i en rekke vanlige matvarer av både animalsk og vegetabilsk opprinnelse, og er også en bestanddel i søtstoffet aspartam. Det finnes ikke data på inntaket av L-aspartat fra kosten i Norge, men data fra USA (NHANES III) viser et gjennomsnittlig inntak på 6,5 g/dag hos voksne. Blant men 31-50 år hadde 99% et inntak på 15,4 g/dag eller lavere.

I litteraturgjennomgangen ble det ikke avdekket noen langtidsstudier med mennesker som var egnet for bruk i risikovurdering av L-aspartat. Det er ikke rapportert om negative helseeffekter fra kortvarige humanstudier med doser fra 1 til 10 g L-aspartat, og varighet fra en enkelt dose opp til 4 uker. Ingen av disse korttidsstudiene hadde imidlertid som hovedmål å avdekke negative helseeffekter.

Det ble funnet to dyrestudier i litteratursøket. En av disse var en 90 dagers subkronisk toksisitetsstudie. I denne studien ble det etablert en NOAEL (no observed adverse effect level) på 697 mg/kg kroppsvekt per dag i hannrotter og 715 mg/kg kroppsvekt per dag i hunnrotter. Nevrotoksiske funn har blitt beskrevet før, men ble ikke funnet i denne studien, det ble imidlertid observert toksiske effekt på nyrer og spyttkjertler ved en dose på 1400 mg/kg kroppsvekt per dag (LOAEL – lowest observed adverse effect level). I VKMs risikokarakterisering av L-aspartat er NOAEL-verdien på 697 mg/kg kroppsvekt per dag lagt til grunn og vurdert opp mot de alternative dosene fra kosttilskudd.

De beregnede verdiene for "margin of exposure" (MOE-verdiene) er i området 5 til 16 for de spesifiserte dosene i kosttilskudd på 3000 til 5700 mg/dag av L-aspartat.

Disse lave MOE-verdiene kan ikke anses som akseptable ettersom L-aspartat har forårsaket toksiske effekter på nyrene (regenerative nyretubuli med tubulær utvidelse) og hypertrofi av acinære celler i spyttkjertler hos rotter. Mulige negative helseeffekter hos mennesker er heller ikke kjent på grunn av mangelen på langtidsstudier.

Vitenskapskomiteen for mattrygghet (VKM) konkluderer med at:

- For voksne (≥18 år), ungdom (14 til <18 år) og barn (10 til <14 år) vil dosene 3000, 3500, 4000, 4500, 5000 og 5700 mg/dag L-aspartat i kosttilskudd kunne representere en risiko for negative helseeffekter.

Barn under 10 år inngår ikke i oppdraget.

Kort sammendrag:

Vitenskapskomiteen for mattrygghet (VKM) har på oppdrag fra Mattilsynet vurdert risiko ved inntak av spesifikke doser av aminosyren L-aspartat i kosttilskudd. VKM konkluderer med at dosene 3000, 3500, 4000, 4500, 5000 og 5700 mg/dag L-aspartat i kosttilskudd vil kunne representere en risiko for negative helseeffekter hos voksne (≥18 år), ungdom (14 til <18 år), og barn (10 til <14 år).

Abbreviations and glossary

Abbreviations

AESAN - Spanish Agency for Food Safety and Nutrition
AFSSA - French Food Safety Agency
ANSES - French Agency for Food, Environmental and Occupational Health and Safety
bw - body weight
EFSA - European Food Safety Authority
JECFA - Joint FAO/WHO Expert Committee on Food Additives
IOM - Institute of Medicine, USA
LOAEL - lowest observed adverse effect level
MOE - Margin of exposure
NFSA - Norwegian Food Safety Authority [*Norw.*: Mattilsynet]
NHANES - National Health and Nutrition Examination Survey
NOAEL - no observed adverse effect level
PLP - pyridoxal phosphate
SCF - Scientific Committee on Food
UL - tolerable upper intake level
VKM - Norwegian Scientific Committee for Food Safety [Norw.: Vitenskapskomiteen for Mattrygghet]
WHO - World Health Organization
XAG - Na(+)-dependent glutamate transport system

Glossary

"Other substances": a substance other than a vitamin or mineral that has a nutritional or physiological effect (European Regulation (EC) No. 1925/2006, Article 2; http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R1925&from=en).

"Negative health effect" and "adverse health effect" are broad terms. VKM uses the definition endorsed by EFSA 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 (EFSA, 2006; WHO, 1994).

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).

The Norwegian Food Safety Authority (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. In preparation for a regulation, 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, flavourings, 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) has requested the Norwegian Scientific Committee for Food Safety (VKM) to assess the safety of L-aspartic acid in food supplements at the following doses: 3000, 3500, 4000, 4500, 5000 and 5700 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). The safety assessments of "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 and/or physiological effect*, and may be added to food supplements or e.g. energy drinks.

This risk assessment regards the substance L-aspartic acid (aspartate) *per se*, and no specific products.

VKM has in this series of risk assessments of "other substances" not evaluated documentation of 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-aspartic acid is an ingredient in food supplements purchased in Norway. NFSA has requested a risk assessment of the intake of 3000, 3500, 4000, 4500, 5000 and 5700 mg L-aspartic acid per day from food supplements. The total L-aspartic acid exposure from other sources than food supplements, such as foods and cosmetic products, is not included in the risk assessment. A dietary requirement for aspartic acid in healthy humans has not been estimated since it is not considered an essential nutrient. According to the third US National Health and Nutrition Examination Survey (NHANES III, 1988-1994), the overall mean intake of L-aspartic acid from food and food supplements in the United States was 6.5 g/day.

L-aspartic acid is a dispensable dicarboxylic amino acid that can be produced by the transamination of oxaloacetic acid, an intermediate in the metabolism of e.g. glucose and amino acids.

L-aspartic acid is a constituent of most proteins and peptides synthesised in the body. In adults, most of the ingested L-aspartic acid is used as an energy fuel (2.3 kcal/g). L-aspartic acid is also used for the synthesis of L-asparagine, pyrimidine and purine nucleotides, and the neurotransmitter N-methyl D-aspartate (Kohlmeier, 2015).

All foods contain significant amount of L-aspartic acid, thus total protein intake is a major determinant of L-aspartic acid intake. The additive aspartame, used as a sweetener, is an exogenous source of L-aspartic acid, as it metabolises into aspartic acid, phenylalanine and methanol (AESAN, 2015). Aspartame contains about 40% aspartic acid (IOM, 2005).

2 Hazard identification and characterisation

2.1 Literature

This risk assessment is based on previous risk assessments of L-aspartic acid, as well as scientific papers retrieved from two systematic literature searches: one search in literature published before 21 October 2016 aiming at retrieving human studies on adverse effects caused by L-aspartic acid, and one search in literature published before 8 November 2016 aiming at retrieving animal model studies on toxicity of L-aspartic acid.

2.1.1 Previous risk assessments

Risks related to L-aspartic acid have previously been evaluated by the Institute of Medicine (IOM), USA, 2005, and by the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (ASEAN), Spain, 2015. An opinion from EFSA (2013) concerning aspartame as a food additive, also contained information on aspartic acid, and a review of the most important information is presented at the end of this chapter.

Table 2.1.1-1: Overview of previous risk assessments of L-aspartic acid.

Risk assessment body, country, publication year	Objective	Conclusion	Suggested doses
IOM, USA, 2005	To establish dietary reference intakes and identify potential adverse effects of L-aspartic acid and other nutrients	Data were not available for dose-response assessment and derivation of a UL for L-aspartic acid in apparently healthy humans	Dietary supplement doses of up to 8 g/d (approximately 120 mg/kg body weight/d) have not resulted in any documented adverse effects
ASEAN, Spain, 2015	To assess the use of L-aspartic acid as a food supplement	Considering the NOAEL of 700 mg/kg/day and using an uncertainty factor of 100, the ADI (Acceptable Daily Intake) is 7 mg/kg/day	A maximum daily quantity of 490 mg/d of L-aspartic is acceptable from the safety point of view for use as a food supplement

Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids from Institute of Medicine (IOM). USA, 2005

According to the IOM (2005), all human studies on the effects of aspartic acid were of short duration (acute doses ranging from 1 to 10 g/day, for time periods between one single dose

and four weeks). These studies found no adverse health effect, when L-aspartic acid was given in acute doses up to 10 g. There are two subchronic studies with duration up to 13 weeks on the oral administration of aspartame to humans (Frey, 1976; Knopp et al., 1976); however, in both studies no dose-response data were available. The IOM noted that dietary supplement doses up to 8 g/day (approximately 120 mg/kg bw per day) of L-aspartic acid had not resulted in any documented adverse effects. However, the IOM concluded that there were insufficient scientific data to develop a tolerable upper intake level (UL) for aspartic acid, due to lack of dose-response data.

According to the IOM (2005), all animal studies on the effects of aspartic acid involved acute exposures. The most serious endpoint identified in animal studies was the development of neuronal necrosis in the hypothalamus of newborn rodents after oral administration of aspartic acid a few days postpartum. Neuronal necrosis in the hypothalamus was not found in newborn non-human primates with levels of plasma dicarboxylic amino acids 10 times those found in newborn mice with neuronal necrosis (Stegink, 1976; Stegink et al., 1974). In view of the scientific debate regarding the sensitivity of newborn animals to the consumption of supplemental dicarboxylic amino acids, it was concluded that aspartic acid dietary supplements were not advisable for infants and pregnant women.

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. Spain, 2015.

According to AESAN (2015), excess L-aspartic acid may cause neurotoxicity, in particular hypothalamic lesions in newborn rodents (Schainker and Olney, 1974; Tada et al., 2008) carried out a subchronic toxicity study in rats, in which the authors established a no observed adverse effect level (NOAEL) for female rats of 715.2 mg/kg bw per day and of 696.6 mg/kg bw per day for male rats. Considering the NOAEL of 696.6 mg/kg bw per day (700 mg/kg bw per day) and using an uncertainty factor of 100, the acceptable daily intake (ADI) was 7 mg/kg bw per day. For an individual weighing 70 kg, this would represent a daily intake of 490 mg of L-aspartic acid. AESAN (2015) concluded that a maximum daily quantity of 490 mg of L-aspartic was acceptable from the safety point of view for use as a food supplement. In addition, it was noted that L-aspartic acid forms part of the habitual diet and is eaten as a component of the sweetener aspartame.

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

Numerous intervention trials have been performed in adults with different aspartate compounds (sodium, magnesium, potassium-magnesium, buffered aspartic acid, and arginine aspartate) in doses ranging from 1 to 10 g/day, for time periods between one single dose and four weeks. None of these studies were undertaken to assess toxicity of aspartic acid intake. However, excluding reports on plasma amino acid imbalance and soft stools/diarrhea, no other adverse effects were reported. In neonatal rodents aspartate have

caused hypothalamic neuronal death, if given orally in large doses (500 mg/kg bw or higher) to infant animals (Olney, 1969; Olney and Ho, 1970; Lemkey-Johnson and Reynolds, 1974; Okaniwa et al., 1979; Finkelstein et al., 1983; Daabees et al., 1985 all cited in EFSA, 2013). A 90-day L-aspartic acid feeding study was performed by Tada et al., 2008. No signs or symptoms of neurotoxicity were observed at any of the doses tested. A NOAEL of 700 mg/kg bw/day for males was identified. The next higher dose gave renal toxicity findings (an apparent dose related regenerative renal tubules dilation in males accompanied by inflammatory cell infiltration).

2.1.2 Literature search

Two systematic literature searches aiming at publications on adverse effects caused by L-aspartic acid was performed in MEDLINE and EMBASE with no restriction on publication year. Both databases were searched to ensure comprehensive study retrieval. The search for human studies was conducted on 21 October 2016, and the search for animal studies was conducted 8 November, 2016. Both searches were limited to publications in English or Scandinavian languages. Conference abstracts, editorials and letters were not included in the searches. The search strategies are outlined in Appendix 1.

2.1.2.1 Publication selection and data extraction

The literature searches identified 2431 titles; 777 in the search for human studies and 1654 in the search for animal studies. All titles and abstracts were screened against the following inclusion criteria:

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

In vitro studies were not included.

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. Titles and abstracts that did not fulfil the inclusion criteria were excluded. Titles and abstracts of unclear relevance to the current risk assessment were retained for further review. The primary screening was performed independently by two Panel members. The publications that passed the primary screening were reviewed in full text against the same inclusion criteria by the author of this report.

The first screening of titles and abstracts of human studies identified 7 relevant publications for full-text review. Two additional human studies were identified in previous reports. After full-text review, all nine publications were considered not relevant and excluded.

The first screening of titles and abstracts of animal studies identified 2 relevant publications for full-text review. Both of these were found relevant and included after full-text review. (see Figure 2.1.2.1-1).

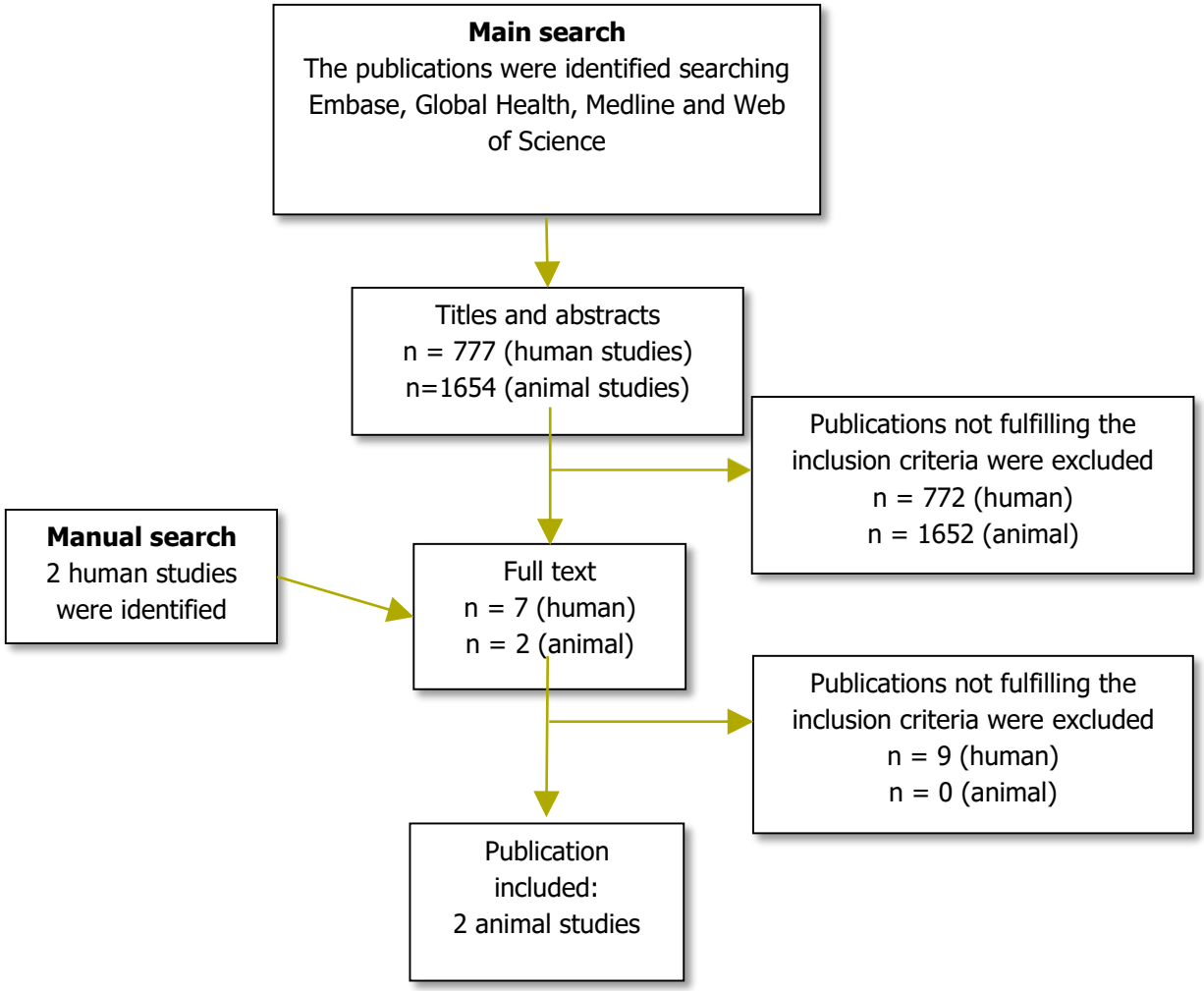

Figure 2.1.2.1-1: Flowchart for publication selection for L-aspartic acid.

2.2 General information

2.2.1 Chemistry

The molecular formula of L-aspartic acid is $C_4H_7NO_4$ and the CAS number is 56-84-8. L-aspartic acid has a molecular weight of 133.1 g/mol. The structural formula is shown in

figure 2.2.1-1. L-Aspartic acid is a dispensable dicarboxylic amino acid that can be produced by the transamination of oxaloacetic acid, an intermediate in the metabolism of e.g. glucose and some amino acids. In the presence of α-ketoglutarate, L-aspartic acid is converted to oxaloacetate and glutamate (Kohlmeier, 2015).

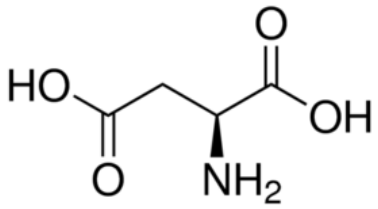

Figure 2.2.1-1: The structural formula of L-aspartic acid.

2.2.2 Occurrence

All protein foods contain significant amounts of L-aspartic acid, thus total protein intake is a major determinant of L-aspartic acid intake. Relatively L-aspartic acid rich proteins are consumed with soybeans (124 mg/g total protein) and other legumes. Fish protein contains approximately 100 mg/g, eggs 100 mg/g, rice 94 mg/g, and most meats around 90 mg/g. Wheat protein contains only 50 mg/g (Kohlmeier, 2015). In the body, the Krebs-cycle intermediate oxaloacetate is the abundantly available precursor for L-aspartic acid. Aspartate aminotransferase uses the amino group from L-glutamate for synthesis of L-aspartic acid (Kohlmeier, 2015). The food sweetener aspartame is an exogenous source of L-aspartic acid, as it hydrolysed to aspartic acid, phenylalanine and minor quantities of methanol in the body (Stegink, 1976). Aspartame contains about 40% aspartic acid (EFSA, 2013).

2.3 Absorption, distribution, metabolism and excretion (ADME)

2.3.1 Absorption and distribution

Free amino acids and small peptides are absorbed nearly completely from the duodenum and jejunum. Free L-aspartic acid can enter intestinal cells via the XAG transport system. Thereafter it is transported to the liver for redistribution in the body. Most L-aspartic acid in blood is bound in proteins, and only minimal amounts circulate as free amino acids (Kohlmeier, 2015).

2.3.2 Metabolism and excretion

The two major catabolic pathways of L-aspartic acid are transamination and utilisation of the resulting oxaloacetate, and utilisation in the urea cycle. A small amount of L-aspartic acid is directly converted to alanine by aspartic 4-decarboxylase in the liver and in the kidneys. In the first reaction, the transamination reaction, the PLP-dependent aspartate aminotransferase moves the amino group from L-aspartic acid to alpha-ketoglutarate,

producing glutamate. Both cytosolic and mitochondrial isoforms are abundant. Quantitatively minor transamination reactions are also catalysed by a few other coenzymes. The second step is the oxidative deamination of glutamate by the enzyme glutamate dehydrogenase, which takes place in the mitochondria. Consequently urea is formed. The oxaloacetate produced in the transamination may serve as a metabolite in the Krebs cycle and used for the production of glucose via gluconeogenesis (Kohlmeier, 2015).

2.4 Toxicological data/Adverse effects

2.4.1 Animal studies

Two relevant animal studies were identified and included in this risk assessment (Table 2.4.1-1).

Table 2.4.1-1: An overview of animal studies investigating L-aspartic acid and adverse health effects.

Reference	Animals	Doses	Main endpoints	Duration of exposure	Adverse effects	NOAEL (mg/kg bw per day)
Schieber et al., (1997)	7 female SPF Sprague-Dawley rats per group	0 (control), 50 mg/kg bw per day in drinking water	Urinalysis, hematology, serum biochemistry, organ weights, gross- and histopathological examination	28 days	None	Not addressed
Tada et al., (2008)	50 male and 50 female Fischer 344 rats (10 per sex per group)	Powder diet containing 0%, 0.05%, 1.25%, 2.5% and 5.0% concentrations of aspartic acid	Urinalysis, hematology, serum biochemistry, organ weights, gross- and histopathological examination	90 days	Toxic effects on kidneys (regenerative renal tubules with tubular dilation) (2.5% and 5.0% in males) Possible acinar cell hypertrophy of the submandibular and parotid glands (2.5% and 5.0% in both sex)	M: 697 F: 715

Evaluation of D-amino acid levels in rat by gas chromatographyselected ion monitoring mass spectrometry: no evidence for subacute toxicity of orally fed D-proline and D-aspartic acid, Schieber et al., 1997

In this 28-day subacute toxicity study, seven Sprague-Dawley rats received deionised water (controls) while seven rats received drinking water with added L-aspartic acid corresponding to a mean daily load of approximately 50 mg L-aspartic acid/kg bw (Schieber et al., 1997).

After 28 days, the weights of the supposed target organs of toxicity (kidney, liver, brain, thymus) were determined and organs were inspected for macroscopic and microscopic alterations. No pathological changes in the organs were observed and no signs of subacute toxicity (liver, kidney) were found. The publication does not include any statement about whether the study was performed in accordance with official guidelines for testing of chemicals in animals.

Toxic effects of L-aspartic acid at high dose levels on kidneys and salivary glands in Fischer 344 rats detected in a 90-day feeding study, Tada et al., 2008

A subchronic oral toxicity study of L-aspartic acid was conducted with groups of 50 male and 50 female Fischer 344 rats fed a powder diet containing 0%, 0.05%, 1.25%, 2.5% and 5.0% concentrations for 90 days (10 animals of each sex received each dose) (Tada et al., 2008). Serum biochemistry showed treatment-related decreases of blood urea nitrogen (2.5% or higher in males and in the 5.0% females), creatinine (5.0% or greater/higher males and of the 1.25% or greater/higher females) and uric acid levels (2.5% male and of the 1.25% or greater/higher females). In addition, incidences of urinary ketone and protein were significantly increased in treated animals of both sexes, while relative kidney weight was significantly increased in the 5.0% male rat. Regenerative renal tubules with tubular dilation were histopathologically observed in male rats of the 2.5% or greater groups. Acinar cell hypertrophy of salivary glands was histopathologically evident in male and female rats of the 2.5% or greater groups. The results indicated that L-aspartic acid causes toxic effects on kidneys and possibly salivary glands at 2.5% and/or higher doses in male and female Fischer 344 rats. The lowest observed adverse effect level (LOAEL) for L-aspartic acid in this study was thus defined by the 2.5% dose and corresponded to a consumed quantity of 1400 mg/kg bw per day. The NOAEL was defined by the 1.25% dose, and corresponded to a consumed quantity of 696.6 mg/kg bw per day for males and 715.2 mg/kg bw per day for females. No signs suggesting neurotoxicity of L-aspartic acid were observed, and a specific neurotoxicity test was therefore not carried out. The authors discussed that neurotoxicity of the amino acid may be limited not only to the species level of rodent but also the age of the newborn.

2.4.1.1 Interactions

There was no information concerning interactions in the literature reviewed in the present 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. L-aspartic acid per se would not be expected to behave as an allergen.

2.4.2 Vulnerable groups

Neonatal rodents are sensitive to the consumption of supplemental dicarboxylic amino acids since they lack the ability to metabolise the dicarboxylic amino acids (Stegink, 1976). The newborn rodent is particularly susceptible to brain lesions, and other dietary substances such as salt and sucrose has also produced brain lesions (Stegink, 1976). Administration of large quantities of glutamate and L-aspartic acid to newborn mice produces a variety of neurotoxic effects, the most marked of which is neuronal necrosis. This finding has, however, not been reproduced in neonatal nonhuman primates by a number of other scientists when giving either glutamate or aspartame at high dosages (EFSA, 2013). However, due to lack of long term studies on L-aspartic acid intake and possible negative health effects in humans, IOM (2005) concluded that aspartic acid dietary supplements are not advisable for infants and pregnant women. Neurotoxic effects of dicarboxylic amino acids in animal species other than newborn rodents are highly controversial, and the available data indicate little relevance to humans. In the present literature review, no studies with L-aspartic acid in children were found. There are no data indicating that children and adolescents are more vulnerable than adults for L-aspartic acid.

2.5 Summary of hazard identification and characterisation

Literature searches including both human and animal studies have been conducted, in addition to reviewing previous reports (IOM, 2005; VKM, 2011). According to IOM (2005), all human and animal studies on the effects of aspartic acid were of short duration and there was a lack of dose-response data. The IOM (2005) therefore concluded that there are not sufficient scientific data to establish a UL for aspartic acid. The IOM (2005) noted that dietary supplement doses of up to 8 g/day (approximately 120 mg/kg bw per day) had not resulted in any documented adverse effects, however no reference was provided for this statement.

Based on the systematic literature searches, we did not identify any long-term studies in healthy individuals that could be used for this risk assessment. In rats, a 90-day subchronic toxicity study by Tada et al. (2008) was identified, reporting a NOAEL of 697 mg/kg bw per day in males and 715 mg/kg bw per day in females. A LOAEL was identified at 1400 mg/kg bw day with toxic effects on the kidneys (regenerative renal tubules dilation accompanied by inflammatory cell infiltration) and acinar cell hypertrophy of salivary glands.

In summary, the following information is considered in the current assessment:

- No long-term studies on L-aspartic acid in healthy children, adolescents or adult humans were found.
- Short-term human studies found no adverse health effect when L-aspartic acid was given in acute doses ranging from 1 to 10 g for time periods between one single dose and four weeks. These studies were however not designed to assess toxicity of L-aspartic acid.
- Administration of large quantities of L-aspartic acid to newborn mice has produced a variety of neurotoxic effects, the most marked of which was neuronal necrosis. Neurotoxic effects of dicarboxylic amino acids in animal species other than newborn rodents are highly controversial, and the available data indicate little relevance for humans.
- A 90-day subchronic toxicity study in rats, with a NOAEL of 697 mg/kg bw per day in males and 715 mg/kg bw per day in females was identified. No neurotoxicity was found, however a toxic effect on kidneys and possibly salivary glands was observed at 1400 mg/kg bw per day (LOAEL).

For the risk characterisation (chapter 4), the NOAEL of 697 mg/kg bw per day derived from the abovementioned subchronic toxicity study in rats is used for comparison with the estimated exposures from food supplements.

3 Exposure / Intake

The exposure to L-aspartic acid was estimated for the doses received by the NFSA for the age groups children (10 to <14 years), adolescents (14 to <18 years) and adults (≥18 years).

3.1 Food supplements

The Norwegian Food Safety Authority requested VKM to perform a risk assessment of 3000, 3500, 4000, 4500, 5000 and 5700 mg/day L-aspartic acid in food supplement for children (10 – 17 years) and adults. The default body weights for age groups determined by EFSA were used: 10 to <14 years = 43.4 kg, 14 to <18 years = 61.3 kg and adults = 70.0 kg. The exposures per kg bw are given in Table 3.1-1.

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

Groups	Daily doses (mg)	Body weight (kg)	Exposure (mg/kg bw per day)
Children (10 to <14 years)	3000, 3500, 4000, 4500, 5000 and 5700	43.4	69, 81, 92, 104, 115 and 131
Adolescents (14 to <18 years)	3000, 3500, 4000, 4500, 5000 and 5700	61.3	49, 57, 65, 73, 82 and 93
Adults (≥18 years)	3000, 3500, 4000, 4500, 5000 and 5700	70.0	43, 50, 57, 64, 71 and 81

3.2 Other sources

Based on the NHANES III (1988-1994), the overall mean intake of L-aspartic acid from food and food supplements in the United States was 6.5 g/day (IOM, 2005). Men 31 through 50 years of age had the highest intake at the 99th percentile of 15.4 g/day.

4 Risk characterisation

The doses received from the NFSA for assessment were 3000, 3500, 4000, 4500, 5000 and 5700 mg/day L-aspartic acid in food supplements, and the estimated exposures for adults, adolescents and children 10 years and older derived from these dose levels are given in chapter 3.

The NOAEL used in this report is 697 mg/kg bw per day derived from a 90-day subchronic toxicity study in rats (Tada et al., 2008). The margins of exposure (MOE) between the NOAEL of 697 mg/kg bw per day and the exposure of L-aspartic acid from food supplements are presented in Table 4-1.

Table 4.-1: The calculated margins between NOAEL (697 mg/kg bw per day) from an animal study, and the exposure to L-aspartic acid from food supplements (MOE-values) for the various age groups.

Age groups	3000 mg/day	3500 mg/day	4000 mg/day	4500 mg/day	5000 mg/day	5700 mg/day
Children (10 to <14 years) (43.4 kg)	10	9	8	7	6	5
Adolescents (14 to <18 years) (61.3 kg)	14	12	11	10	9	8
Adults (≥18 years) (70 kg)	16	14	12	11	10	9

The calculated MOE-values for the NOAEL from the animal study ranged from 5 to 16 for a daily intake of 3000 to 5700 mg/day of L-aspartic acid.

These low MOE-values are not regarded as acceptable since L-aspartic acid has caused toxic effects on the kidneys (regenerative renal tubules with tubular dilation) and acinar cell hypertrophy of salivary glands in rats and direct information on the potential adverse health effects in humans is not available due to an absence of long-term studies.

No studies with L-aspartic acid in children were found. There are no data indicating that children and adolescents are more vulnerable than adults for L-aspartic acid. No tolerance level is set for L-aspartic acid specifically for children or adolescents.

VKM considers that:

In adults (≥18 years), the specified doses 3000, 3500, 4000, 4500, 5000 and 5700 mg/day L-aspartic acid in food supplements may represent a risk of adverse health effects.

In adolescents (14 to <18 years), the specified doses 3000, 3500, 4000, 4500, 5000 and 5700 mg/day L-aspartic acid in food supplements may represent a risk of adverse health effects.

In children (10 to <14 years), the specified doses 3000, 3500, 4000, 4500, 5000 and 5700 mg/day L-aspartic acid in food supplements may represent a risk of adverse health effects.

5 Uncertainties

For the current risk assessment there are potentially large uncertainties arising from:

- The lack of relevant human data available for risk characterisation
- The risk characterisation being based on a 90-day (subchronic) study in rats – species extrapolation
- The lack of knowledge about habitual daily dietary intake of L-aspartic acid in Norway
  The assumption that children and adolescents have similar tolerance as adults relative to their body weight, due to lack of data
- The possible failure of the systematic literature search, based on the predefined search criteria, to identify relevant literature reporting adverse effects of L-aspartic acid in humans or animals

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-aspartic acid in food supplements at the doses 3000, 3500, 4000, 4500, 5000 and 5700 mg/day for the general population, ages 10 years and above. The specific doses were not regarded as acceptable since L-aspartic acid has caused toxic effects on the kidneys (regenerative renal tubules with tubular dilation) and acinar cell hypertrophy of salivary glands in rats and information of the potential adverse health effects in human is unknown due to lack of long-term studies.

VKM concludes that:

- In adults (≥18 years), the specified doses 3000, 3500, 4000, 4500, 5000 and 5700 mg/day L-aspartic acid in food supplements may represent a risk of adverse health effects.
- In adolescents (14 to <18 years), the specified doses 3000, 3500, 4000, 4500, 5000 and 5700 mg/day L-aspartic acid in food supplements may represent a risk of adverse health effects.
- In children (10 to <14 years), the specified doses 3000, 3500, 4000, 4500, 5000 and 5700 mg/day L-aspartic acid in food supplements may represent a risk of adverse health effects.

An overview of the conclusions is presented in Table 6-1.

Table 6-1 An overview of the conclusions on L-aspartic acid in food supplements. Red: estimated exposure may represent a risk of adverse health effects.

	L-aspartic acid					
Food supplement **Age groups**	3000 mg/day	3500 mg/day	4000 mg/day	4500 mg/day	5000 mg/day	5700 mg/day
Children (10 to <14 years)						
Adolescents (14 to <18 years)						
Adults (≥18 years)						

7 Data gaps

- There is a lack of long-term studies on L-aspartic acid in healthy children, adolescents and adult humans
- There are few toxicological studies in animals where L-aspartic acid is provided as a single supplement and with an appropriate study design to investigate possible long-term adverse effects

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9 Appendix

Search strategy for human studies

Database: Embase <1974 to 2016 October 20>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present>

1. aspartic acid*.ti. (4359)
2. aspartat*.ti. (23958)
3. 1 or 2 (28272)
4. (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. (10379211)
5. 3 and 4 (5506)
6. (conference abstract* or letter* or editorial*).pt. (5202828)
7. 5 not 6 (5333)
8. limit 7 to (danish or english or norwegian or swedish) (5198)
9. limit 8 to human (1263)
10. remove duplicates from 9 (777)

Search strategy for animal studies

1. Database: Embase <1974 to 2016 November 07>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present>
2. aspartic acid*.ti. (4356)
3. aspartat*.ti. (23917)
4. 1 or 2 (28228)
5. (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. (10411790)
6. 3 and 4 (5511)
7. (conference abstract* or letter* or editorial*).pt. (5215921)
8. 5 not 6 (5331)
9. limit 5 to (danish or english or norwegian or swedish) (5376)
10. limit 8 to animals (2694)
11. remove duplicates from 9 (1654)