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Risk assessment of "other substances" – L-glutamine and L-glutamic 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) 2016: 25
Risk assessment of “other substances” – L-glutamine and L-glutamic acid

Opinion of the Panel on Nutrition, Dietetic Products, Novel Food and Allergy of the Norwegian Scientific Committee for Food Safety
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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 the NFSA. These risk assessments will provide the NFSA with the scientific basis for 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 or physiological effect*. They are 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 limited to the use of L-glutamine and L-glutamic acid in food supplements. Risks related to glutamine and glutamic acid added to food and drinks, protein hydrolysates or high dietary protein intake are outside the scope of the opinion. The report is based on previous risk assessments of glutamine and glutamic acid and scientific papers retrieved from a comprehensive literature search.

L-glutamine is considered a non-essential amino acid in humans. In addition to its role in protein synthesis and the handling by the body of ammonia (via urea cycle), L-glutamine participates in other complex metabolic pathways e.g. in the central nervous system, immune system, and insulin secretion. L-glutamine is deaminated by glutaminase to form glutamic acid. L-glutamine is available from all protein-containing foods. High-protein foods contain the most (e.g. meat, fish, eggs and dairy products).

L-glutamic acid is a non-essential amino acid. At physiological conditions its side chain is fully ionised, i.e. it exists in the form of glutamate. In addition to its role as substrate in protein synthesis, glutamic acid has important metabolic roles as a source of α-ketoglutarate in the citric acid cycle and in the handling by the body of ammonia (via urea cycle). Glutamic acid is also a major neurotransmitter. In the unbound form only, glutamic acid is responsible for umami, one of the five basic tastes sensed by humans. Glutamic acid is used as a flavour enhancer in the form of its salt monosodium glutamate. All meats, poultry, fish, eggs, and dairy products are excellent sources of glutamic acid. Some protein-rich plant foods also serve as sources, e.g. wheat protein contains 30% to 35% glutamic acid.

According to information from the NFSA, L-glutamine and glutamic acid are ingredients in food supplements sold in Norway. The NFSA has requested a risk assessment of the following doses of L-glutamine in food supplements: 3500 mg/day, 5000 mg/day, 8000 mg/day, 10000 mg/day, 12000 mg/day, 15000 mg/day, and 16500 mg/day, and the following doses of glutamic acid: 1000 mg/day, 2000 mg/day, 3000 mg/day, 4000 mg/day, 5000 mg/day, and 5500 mg/day. Dietary intake in Norway is not known, but data from the

third National Health and Nutrition Examination Survey (NHANES III) 1988-1994 in the USA suggest a mean dietary intake of about 15 g glutamic acid per day.

In phase 1 of the present evaluation of "other substances", previous reports that assessed the safety of L-glutamine or L-glutamic acid supplementation in humans were identified. For the present report, a systematic literature search was performed to retrieve human studies published in the period 2011-2015, and in addition separate literature searches were performed for animal studies and studies in children and adolescents. The main search retrieved no publications reporting results from trials with L-glutamine or L-glutamic acid in healthy humans, nor did the search for studies in children and adolescents identify any relevant publications. Three human studies on glutamates were included as part of the risk assessment of glutamic acid. The search for animal studies retrieved four relevant reports.

No major specific issues related to safety of L-glutamine and L-glutamic acid used as food supplements were identified in previous reports. However, a lack of studies in healthy adult individuals as well as in children was pointed out, and in particular the absence of long-term studies in healthy individuals.

According to previous reports, short-term intake of doses of L-glutamine up to 0.5 g/kg bw per day has not been found to cause significant adverse effects. Up to 1.5 g per day of L-glutamic acid has been reported not to be associated with adverse effects. Conclusions in previous reports have indicated maximum supplemental levels of 3.5 and 5 g per day of L-glutamine and 1 g per day of L-glutamic acid.

For the risk characterisation of L-glutamine, in the absence of long-term human studies in healthy individuals, VKM will base the value of comparison on the highest dose tested (no observed adverse effect level; NOAEL) in two 90-day studies in rodents, 3832 mg/kg bw per day. Employing an uncertainty factor of 10 for the extrapolation between species, the value of comparison is set to 383 mg/kg bw per day, corresponding to 26.8 g per day in a 70 kg adult. Data from studies in various patient groups support the data from the two animal studies indicating the absence of significant adverse effects with this dose.

In the risk characterisation of L-glutamic acid, in the absence of any unequivocally demonstrated reproducible adverse effect in short-term human studies and an absence of long-term studies in healthy individuals, VKM will base the value of comparison on the highest dose tested (NOAEL) in a 28-day study in rodents, 953 mg/kg bw per day. Employing an uncertainty factor of 10 for the extrapolation between species, the value of comparison is set to 95 mg/kg bw, corresponding to 6.7 g per day in a 70 kg adult. Data from early long-term studies in humans (doses up to 45 g per day) and in animals as well as short-term studies on glutamates support the data from the animal study indicating the absence of significant adverse effects with this dose.

Based on these data, the Norwegian Scientific Committee for Food Safety (VKM) concludes that:

L-glutamine

- In adults (≥18 years), the specified doses of 3500, 5000, 8000, 10000, 12000, 15000 and 16500 mg/day L-glutamine in food supplements are considered unlikely to cause adverse health effects.
- In adolescents (14 to <18 years), the specified doses of 3500, 5000, 8000, 10000, 12000, 15000 and 16500 mg/day mg/day L-glutamine in food supplements are considered unlikely to cause adverse health effects.
- In children (10 to <14 years), the specified doses of 3500, 5000, 8000, 10000, 12000, 15000 and 16500 mg/day mg/day L-glutamine in food supplements are considered unlikely to cause adverse health effects.

L-glutamic acid

- In adults (≥18 years), the specified doses of 1000, 2000, 3000, 4000, 5000 and 5500 mg/day L-glutamic acid in food supplements are considered unlikely to cause adverse health effects.
- In adolescents (14 to <18 years), the specified doses of 1000, 2000, 3000, 4000, 5000 and 5500 mg/day L-glutamic acid in food supplements are considered unlikely to cause adverse health effects.
- In children (10 to <14 years), the specified doses 1000, 2000, 3000, and 4000 mg/day L-glutamic acid in food supplements are considered unlikely to cause adverse health effects. The specified doses of 5000 and 5500 mg/day may represent a risk of adverse health effects.

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

Short summary:

The Norwegian Scientific Committee for Food Safety (VKM) has, at the request of the Norwegian Food Safety Authority, assessed the risk of specified doses of L-glutamine and L-glutamic acid in food supplements. VKM concludes that:

L-glutamine

- In adults (≥18 years), the specified doses of 3500, 5000, 8000, 10000, 12000, 15000 and 16500 mg/day L-glutamine in food supplements are considered unlikely to cause adverse health effects.
- In adolescents (14 to <18 years), the specified doses of 3500, 5000, 8000, 10000, 12000, 15000 and 16500 mg/day L-glutamine in food supplements are considered unlikely to cause adverse health effects.

- In children (10 to <14 years), the specified doses of 3500, 5000, 8000, 10000, 12000, 15000 and 16500 mg/day L-glutamine in food supplements are considered unlikely to cause adverse health effects.

L-glutamic acid

- In adults (≥18 years), the specified doses of 1000, 2000, 3000, 4000, 5000 and 5500 mg/day L-glutamic acid in food supplements are considered unlikely to cause adverse health effects.

- In adolescents (14 to <18 years), the specified doses of 1000, 2000, 3000, 4000, 5000 and 5500 mg/day L-glutamic acid in food supplements are considered unlikely to cause adverse health effects.

- In children (10 to <14 years), the specified doses 1000, 2000, 3000, and 4000 mg/day L-glutamic acid in food supplements are considered unlikely to cause adverse health effects. The specified doses of 5000 and 5500 mg/day may represent a risk of adverse health effects.

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

Key words: Adverse health effect, L-glutamine, L-glutamic acid, 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. Disse risikovurderingene vil gi Mattilsynet vitenskapelig grunnlag for å regulere "andre stoffer" i kosttilskudd.

"Andre stoffer" er beskrevet i kosttilskuddirektivet (2002/46/EF) som 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 vurdert potensielle gunstige helseeffekter, men kun vurdert mulige negative helseeffekter.

Denne risikovurderingen er avgrenset til glutamin og glutaminsyre i kosttilskudd. Risiko forbundet med glutamin og glutaminsyre tilsatt i mat og drikke, fra proteinhydrolysater eller fra et høyt proteininntak, er ikke omfattet av denne risikovurderingen. Vurderingen er basert på andre tidligere risikovurderinger av aminosyrene og vitenskapelige artikler som er funnet i systematiske litteratursøk.

L-glutamin er en ikke-essensiell aminosyre for mennesker. I tillegg til å ha en rolle i proteinsyntesen og kroppens håndtering av ammoniakk (via ureasyklus), inngår L-glutamin i andre komplekse metabolske veier, f.eks i sentralnervesystemet , immunsystemet og i insulinsekresjonen. L-glutamin deamineres av glutaminase til glutaminsyre. L-glutamin forekommer i alle proteinholdige matvarer. Matvarer med høyt innhold av protein inneholder mest glutamin (for eksempel kjøtt, fisk, egg og melkeprodukter).

L-glutaminsyre er også en ikke-essensiell aminosyre for mennesker. Under fysiologiske betingelser vil sidekjeden være fullstendig ionisert, dvs. at den forekommer som glutamat. I tillegg til å være substrat i proteinsyntesen, har glutaminsyre viktige metabolske funksjoner som kilde for α-ketoglutarat i sitronsyresyklusen og i kroppens håndtering av ammoniakk (via ureasyklus). Glutamat er også en viktig nevrotransmitter i sentralnervesystemet. I ubunden form gir glutaminsyre opphav til umami, en av de fem grunnleggende smakskvalitetene som menneskenes sanseapparat oppfatter. Glutaminsyre blir derfor benyttet som smaksforsterker i form av sitt salt natriumglutamat, kjent under betegnelsen MSG (eng. *monosodium glutamate*). Kjøtt, fjærkre, fisk, egg og melkeprodukter er gode kilder til glutaminsyre. Noen proteinrike kornsorter kan også være gode kilder, hveteprotein inneholder f.eks. 30 til 35 % glutaminsyre.

Ifølge informasjon fra Mattilsynet er glutamin og glutaminsyre ingredienser i kosttilskudd som selges i Norge. Oppdraget fra Mattilsynet var å risikovurdere følgende doser i kosttilskudd: L-glutamin 3500, 5000, 8000, 10000, 12000, 15000 og 16500 mg/dag, og følgende doser av glutaminsyre: 1000, 2000, 3000, 4000, 5000 og 5500 mg/dag. Vi har ikke data for inntak fra kosten i Norge, men data fra NHANES (USA) i 1988-1994 viste et gjennomsnittlig inntak på ca 15 g glutaminsyre per dag.

I fase 1 ble det identifisert tidligere rapporter som har risikovurdert tilskudd med L-glutamin eller L-glutaminsyre hos mennesker. For denne risikovurderingen er det i tillegg gjort et systematisk litteratursøk for å identifisere humanstudier publisert i perioden 2011-2015. I tillegg er det gjort egne litteratursøk for å identifisere dyrestudier og studier spesielt med barn og ungdom. I hovedsøket ble det ikke funnet noen relevante publikasjoner med L-glutamin eller L-glutaminsyre hos friske mennesker. Heller ikke søket spesielt rettet mot studier blant barn og ungdom ga noen relevante publikasjoner. Tre humanstudier med glutamat ble inkludert som en del av risikovurderingen av glutaminsyre. I søket etter dyrestudier ble det identifisert fire relevante artikler.

Ingen særskilt risiko knyttet til bruk av L-glutamin og L-glutaminsyre som kosttilskudd har blitt beskrevet i tidligere rapporter. Imidlertid er det påpekt mangel på studier hos friske voksne individer så vel som hos barn, og særlig mangel på langtidsstudier hos friske individer.

Ifølge tidligere rapporter har ikke inntak av doser L-glutamin opp til 0,5 g/kg kroppsvekt per dag over kort tid medført bivirkninger av betydning. Opptil 1,5 g per dag av L-glutaminsyre er blitt rapportert å ikke være forbundet med negative helseeffekter. Konklusjoner i tidligere rapporter har angitt doser på 3,5 og 5 g per dag for L-glutamin og 1 g per dag for L-glutaminsyre som trygge nivåer for kosttilskudd.

Til risikovurderingen av L-glutamin har VKM, i fravær av humane langtidsstudier hos friske individer, basert "value for comparison" på en NOAEL (no observed adverse effect level; den høyeste dosen testet) fra to 90-dagers studier i gnagere, 3832 mg/kg kroppsvekt per dag. Med anvendelse av en sikkerhetsfaktor på 10 for ekstrapolering mellom arter, er "value for comparison" satt til 26.8 g per dag for en 70 kg voksen (383 mg/kg kroppsvekt). Data fra studier i ulike pasientgrupper understøtter data fra de to dyrestudiene og indikerer at det ikke forekommer negative helseeffekter av betydning med denne dosen.

Til risikovurderingen av L-glutaminsyre har VKM, i fravær av entydig dokumenterte reproduserbare negative helseeffekter i humane korttidsstudier og langtidsstudier hos friske individer, basert "value for comparison" på en NOAEL (den høyeste dosen testet) fra en 28-dagers studie i gnagere, 953 mg/kg kroppsvekt. Med anvendelse av en sikkerhetsfaktor på 10 for ekstrapolering mellom arter, er "value for comparison" er satt til 6,7 g per dag for en 70 kg voksen (95 mg/kg kroppsvekt). Data fra eldre langtidsstudier med mennesker (doser på opptil 45 g per dag) og eldre dyrestudier understøtter denne NOAELen. Også humane korttidsstudier med glutamat indikerer at det ikke forekommer negative helseeffekter av betydning med den angitte dosen.

Vitenskapskomiteen for mattrygghet (VKM) konkluderer med at:

L-glutamin

- For voksne (≥18 år) er det usannsynlig at de spesifiserte dosene på 3500, 5000, 8000, 10000, 12000, 15000 og 16500 mg/dag glutamin i kosttilskudd vil forårsake negative helseeffekter.
- For ungdom (14 til <18 år) er det usannsynlig at de spesifiserte dosene på 3500, 5000, 8000, 10000, 12000, 15000 og 16500 mg/dag glutamin i kosttilskudd vil forårsake negative helseeffekter.
- For barn (10 til <14 år) er det usannsynlig at de spesifiserte dosene på 3500, 5000, 8000, 10000, 12000, 15000 og 16500 mg/dag glutamin i kosttilskudd vil forårsake negative helseeffekter.

L-glutaminsyre

- For voksne (≥18 år) er det usannsynlig at de spesifiserte dosene på 1000, 2000, 3000, 4000, 5000 og 5500 mg/dag L-glutaminsyre i kosttilskudd vil forårsake negative helseeffekter.
- For ungdom (14 til <18 år) er det usannsynlig at de spesifiserte dosene på 1000, 2000, 3000, 4000, 5000 og 5500 mg/dag L-glutaminsyre i kosttilskudd vil forårsake negative helseeffekter.
- For barn (10 til <14 år) er det usannsynlig at de spesifiserte dosene på 1000, 2000, 3000 og 4000 mg/dag L-glutaminsyre i kosttilskudd vil forårsake negative helseeffekter. Dosene 5000 og 5500 mg/dag L-glutaminsyre i kosttilskudd vil kunne representere en risiko for negative helseeffekter.

Barn under 10 år inngår ikke i dette oppdraget.

Kort sammendrag

Vitenskapskomiteen for mattrygghet (VKM) har på oppdrag for Mattilsynet vurdert risiko ved inntak av spesifikke doser av L-glutamin og L-glutaminsyre i kosttilskudd. VKM konkluderer med at:

L-glutamin

- For voksne (≥18 år) er det usannsynlig at de spesifiserte dosene på 3500, 5000, 8000, 10000, 12000, 15000 og 16500 mg/dag glutamin i kosttilskudd vil forårsake negative helseeffekter.
- For ungdom (14 til <18 år) er det usannsynlig at de spesifiserte dosene på 3500, 5000, 8000, 10000, 12000, 15000 og 16500 mg/dag glutamin i kosttilskudd vil forårsake negative helseeffekter.
- For barn (10 til <14 år) er det usannsynlig at de spesifiserte dosene på 3500, 5000, 8000, 10000, 12000, 15000 og 16500 mg/dag glutamin i kosttilskudd vil forårsake negative helseeffekter.

L-glutaminsyre

- For voksne (≥18 år) er det usannsynlig at de spesifiserte dosene på 1000, 2000, 3000, 4000, 5000 og 5500 mg/dag L-glutaminsyre i kosttilskudd vil forårsake negative helseeffekter.
- For ungdom (14 til <18 år) er det usannsynlig at de spesifiserte dosene på 1000, 2000, 3000, 4000, 5000 og 5500 mg/dag L-glutaminsyre i kosttilskudd vil forårsake negative helseeffekter.
- For barn (10 til <14 år) er det usannsynlig at de spesifiserte dosene på 1000, 2000, 3000 og 4000 mg/dag L-glutaminsyre i kosttilskudd vil forårsake negative helseeffekter. Dosene 5000 og 5500 mg/dag L-glutaminsyre i kosttilskudd vil kunne representere en risiko for negative helseeffekter.

Abbreviations and glossary

Abbreviations

ADI - acceptable daily intake
AESAN - Spanish Agency for Food Safety and Nutrition
AFSSA - French Food Safety Agency (up to 1st July 2010)
ANSES - French Agency for Food, Environmental and Occupational Health and Safety (since 1st July 2010)
bw - body weight
CAS number - chemical abstract service number
CNS - central nervous system
DNA - deoxyribonucleic acid
EFSA - European Food Safety Authority
FAO - Food and Agriculture Organization
FDA - Food and Drug Administration, USA
FEV - forced expiratory volume
GLP - good laboratory practice
GLP-1 - glucagon-like peptide-1
GABA - gamma-aminobutyric acid
GI - gastrointestinal
ICR - Institute of Cancer Research
IOM - Institute of Medicine, USA
JECFA - Joint FAO/WHO Expert Committee on Food Additives
LD_{50} - lethal dose for 50% of the animals
LOAEL - lowest observed adverse effect level
NADP - nicotinamide adenine dinucleotide phosphate
NFSA - Norwegian Food Safety Authority [*Norw.*: Mattilsynet]
NHANES - National Health And Nutrition Examination Survey
NOAEL - no observed adverse effect level
OECD - Organisation for Economic Co-operation and Development
RCT - randomised controlled trial
SAE - serious adverse event
SIT - sitagliptin
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, 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 established by World Health Organization (WHO) 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).

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-glutamine in food supplements at the following doses:
L-glutamine: 3500, 5000, 8000, 10000, 12000, 15000 and 16500 mg/day
L-glutamic acid: 1000, 2000, 3000, 4000, 5000 and 5500 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. VKM has in this series of risk assessments of "other substances" not evaluated any potential beneficial effects from these substances, but merely possible adverse effects at specified doses used in Norway.

This risk assessment regards the substances L-glutamine and L-glutamic acid *per se*, and no specific products.

According to information from the Norwegian Food Safety Authority (NFSA), L-glutamine and L-glutamic acid are ingredients in food supplements purchased in Norway. NFSA has requested a risk assessment of the following doses of L-glutamine from food supplements: 3500, 5000, 8000, 10000, 12000, 15000 and 16500 mg/day, and the following doses of L-glutamic acid from food supplements: 1000, 2000, 3000, 4000, 5000 and 5500 mg/day. Estimated from NHANES III conducted in 1988-1994, mean glutamic acid intake for the US adult population was approximately 15 g/day, while men 31 through 50 years had the highest reported intake at the 99th percentile of 33.7 g/day (IOM, 2005). No data were available for glutamine intake. Information on habitual dietary intake of L-glutamine and L-glutamic acid in Norway is not available.

L-glutamine

L-glutamine is characterised as a non-essential amino acid, and is available from a wide range of protein-rich foods in the normal diet. Endogenous production of glutamine is estimated to 60 -100 mg/day (van Acker et al., 1999).

Besides its proteogenic role, glutamine plays a major role in the body's handling of ammonia (via urea cycle), and participates in other complex metabolic processes e.g. in the central nervous system (CNS), intestine, immune system, and insulin secretion.

Glutamine is synthesised by the enzyme glutamine synthetase from glutamic acid and ammonia, and is hydrolysed by glutaminase to glutamic acid and ammonia.

L-glutamic acid

L-glutamic acid is a non-essential amino acid, available from a wide range of protein-rich foods in the normal diet.

At physiological conditions glutamic acid's side chain is fully ionised, i.e. it exists in the form of glutamate. In addition to its role in protein synthesis, glutamic acid has a central role in energy metabolism (via citric acid cycle) and the body's handling of ammonia (via urea cycle). Glutamic acid is an important excitatory neurotransmitter in the CNS, and is also a precursor for the inhibitory transmitter gamma-aminobutyric acid (GABA).

2 Hazard identification and characterisation

2.1 Literature

The present risk assessment is based on previous risk assessments of L-glutamine and L-glutamic acid including glutamates, and on evidence extracted from articles retrieved in a comprehensive literature search.

2.1.1 Previous risk assessments

Risks related to L-glutamine have previously been evaluated by; Institute of Medicine (IOM), USA, 2005; JECFA (Joint FAO/WHO Expert Committee on Food Additives), WHO Food Additives Series 54, JECFA, 2006; AFSSA (The French Food Safety Agency), 2007; ANSES (The French Agency for Food, Environmental and Occupational Health & Safety), 2011; the Norwegian Scientific Committee for Food Safety (VKM), Norway, 2011; AESAN (The Scientific Committee of the Spanish Agency for Food Safety and Nutrition), 2012; AESAN (The Scientific Committee of the Spanish Agency for Food Safety and Nutrition), 2013.

Risks related to L-glutamic acid have previously been evaluated by Institute of Medicine (IOM), USA, 2005; JECFA (Joint FAO/WHO Expert Committee on Food Additives), WHO Food Additives Series 54, JECFA, 2006; AFSSA (The French Food Safety Agency), 2007; AESAN (The Scientific Committee of the Spanish Agency for Food Safety and Nutrition), 2012.

Of the reports mentioned above, a literature search underlying the report is described only in VKM (2011).

These reports are summarised in Table 2.1.1-1.

Table 2.1.1-1: Overview of previous risk assessments of L-glutamine and L-glutamic acid

Risk assessment body, country, publication year	Objective	Conclusion	Suggested doses
IOM, USA 2005	To establish dietary reference intakes for L-glutamine and L-glutamic acid, and other nutrients. Includes a discussion of potential toxicity.	L-glutamine: The data on supplements are conflicting and are not sufficient for a dose-response assessment and derivation of a tolerable upper intake level (UL). Glutamic acid: A UL for L-glutamate from supplements cannot be established at the present time.	L-glutamine: Not established L-glutamic acid: Not established

Risk assessment body, country, publication year	Objective	Conclusion	Suggested doses
JECFA (Joint FAO/WHO Expert Committee on Food Additives), WHO Food Additives Series 54, WHO, 2006	To evaluate the safety of a group of 20 flavouring agents, including L-glutamine and L-glutamic acid.	The 11 α-amino acids are macronutrients and normal components of protein, hence the use of these substances would not raise any safety concerns at estimated current intakes. The 'ADI not specified' for L-glutamic acid was maintained. Conclusion: L-glutamine: No safety concern L-glutamic acid: No safety concern	L-glutamine: Not established L-glutamic acid: Not established
AFSSA, France, 2007	As one of several tasks to assess minimum and maximum protein and amino acid intake levels in diets in different situations and for different populations	A tolerable upper intake level is not proposed for either nitrogen or amino acids, due to a lack of experimental and epidemiological data.	L-glutamate: Not established L-glutamic acid: Not established
ANSES, France, 2011	To consider whether use of substances with nutritional or physiological effects in foods should be restricted or prohibited	Metabolic effects are expected to be silent at first, but can then reveal themselves in the medium to long term. The complexity of amino acid metabolism and the scarcity of toxicological data do not allow a proper risk assessment. No conclusion drawn.	Glutamine: Not established
VKM, Norway, 2011	To qualitatively rank 30 amino acids according to high, medium or low risk	Glutamine was grouped as "low risk"	Glutamine: Not established
AESAN, Spain, 2012	The use of glutamine as a food supplement was assessed	No adverse effects have been observed with L-glutamine. Although the safety of L-glutamine has not been assessed in healthy subjects or in chronic administration, the proposed dose is found acceptable from a safety point of view.	L-glutamine 2 g/day acceptable
AESAN, Spain, 2012	The use of glutamic acid as a food supplement was assessed	L-glutamic acid is present in foods in the diet, and adverse effects are only observed at doses above 1.5 g. 1 g/day is acceptable from a safety point of view for use in food supplements.	L-glutamic acid 1 g/day acceptable
AESAN, Spain, 2013	The use of glutamine as a food supplement was assessed	No adverse effects have been observed with L-glutamine. Although the safety of L-glutamine has not been assessed in healthy subjects or in chronic administrations, based on available information and general considerations, the proposed dose is found acceptable from a safety point of view.	L-glutamine 5 g daily acceptable

Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients)Chapter 10 "Protein and Amino Acids. Institute of Medicine (IOM). USA, 2005

L-glutamine: IOM (2005) states that despite a substantial number of published investigations in which glutamine has been administered to humans, very few, if any, adverse effects have been reported. In two cited studies with serial assessment of mental status in patients on total parenteral nutrition, no evidence for neurotoxicity was found. However, the IOM states

that the published studies of toxicity have not fully taken into account a number of important factors, including the chronic consumption of glutamine. The issue of tumor growth promotion by glutamine is referred to, but it is stated that the evidence points to the contrary of tumor promotion. In vivo studies are referred to that have not confirmed the suspicion of tumor promotion, oral administration of glutamine in rats did not enhance tumor growth in vivo, and based on available studies glutamine may even depress tumor growth according to the IOM. A study is cited that reported a reversible increase in liver enzymes in patients on total parenteral nutrition supplemented with glutamine 285 mg/kg per day (about 20 g per day), along with other studies reporting no adverse effects.

In conclusion, the data on L-glutamine from supplements were found to be conflicting and not sufficient for a dose-response assessment and derivation of a UL.

L-glutamic acid: The IOM discussion on glutamic acid, including its sodium salt, indicates that longer-term (up to two years) investigations of glutamate in animals have revealed few adverse effects, and a number of negative studies are cited. Studies on chronic glutamate treatment of children with approximately 0.3 g/day of glutamic acid for 6 months (Zimmerman and Burgemeister, 1959) and adults with 45 g/day for 10 weeks (Himwich and Petersen, 1954) showed no adverse effects. Neurotoxic effects of glutamate are discussed at length, but the conclusion is that no signs of neurological damage had been reported in humans. Similarly, a concern that large doses of glutamate taken orally might stimulate the secretion of prolactin and cortisol and inhibit the release of growth hormone had more or less been removed by a more recent and strictly controlled study (Fernstrom et al., 1996).

The so-called Chinese restaurant Syndrome, also called MSG (monosodium glutamate) Symptom Complex (burning sensation at the back of the neck, forearms and chest; facial pressure or tightness; chest pain; headache; nausea; upper body tingling and weakness; palpitation; numbness in the back of the neck, arms and back; and drowsiness) was for a period attributed to high concentrations of MSG in Asian food. However, later, properly conducted and controlled studies failed to establish a relationship between Chinese Restaurant Syndrome and ingestion of MSG (JECFA, 1988). FASEB (1995) similarly concluded that there was no scientifically verifiable evidence for adverse effects in individuals exposed to high levels of MSG. However, FASEB also concluded that there was sufficient evidence for the existence of a small subgroup of healthy people that are sensitive to MSG, showing symptoms when exposed to an oral dose of 3 g in the absence of food. A double blind, placebo-controlled study of self-selected individuals seemed to confirm this (Yang et al., 1997), identifying a dose of 2.5 g as the threshold for induction of symptoms. A later multicenter double blind placebo controlled study by Geha et al. (2000) gave similar findings of more symtoms in the high-dose (5 g) glutamate group than in the placebo group. However, it is also reported that the symptoms did not occur when glutamate was given with food, nor were the symptoms consistent or reproducible in four consecutive challenges within the same individuals. It was noted that neither persistent nor serious effects from glutamate were observed.

IOM further discusses the claimed triggering of asthma by MSG (another facet of the Chinese Restaurant Syndrome), but concludes that although there is a need for further studies to clarify inconsistencies, later double-blind studies did not confirm the precipitation of asthma attacks by MSG. Overall, according to IOM, the publications show no convincing evidence that glutamate triggers asthma attacks. Regarding claims that glutamate exacerbates urticaria, double-blind challenges suggested that only a very small proportion of patients, if any, were sensitive to MSG.

IOM concluded that in relation to glutamic acid including its sodium salt, a tolerable upper intake level (UL) for L-glutamate from supplements could not be established at the present time.

JECFA Joint FAO/WHO Expert Committee on Food Additives Safety evaluation of certain food additives. Prepared by the Sixty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) WHO Food Additives Series: 54, 2006

A statement is made that "In view of the fact that the L-form of the 11 α-amino acids and the one α-imino acid in this group are macronutrients and normal components of protein, the use of these substances would not raise any safety concerns at estimated current intakes" (JECFA, 2006).

The acceptable daily intake (ADI) assessment "not specified" for L-glutamic acid was maintained. Conclusions: L-glutamine: No safety concern; L-glutamic acid: No safety concern.

Protein intake: dietary intake, quality, requirements and recommendations (A French and English executive overview of the report). Agence Francaise de Sécurité Sanitaire des Aliments (AFSSA). France, 2007

In this report, a tolerable upper intake level is not proposed for either nitrogen or amino acids, due to a lack of experimental and epidemiological data (AFSSA, 2007).

Opinion of the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) on the assessment of the risks associated with substances with nutritional or physiological effects with a view to restricting or prohibiting their use in foodstuffs. France, 2011

It is stated that glutamine has quite good tolerance in humans. The complexity of amino acid metabolism and the scarcity of toxicological data do not allow a proper risk assessment. No conclusion was drawn (ANSES, 2011).

VKM report on risk categorisation of amino acids. Norway, 2011

In 2011, VKM conducted a risk categorisation of about 30 amino acids and amino acid compounds based on potential health risks related to high intakes of the amino acids (VKM, 2011). It was emphasised that the VKM report from 2011 has several limitations and can only be regarded as an initial screening and not as risk assessment of the many amino acids.

L-glutamine: The task was to evaluate individual amino acids to place them into one of three groups: high, moderate or low potential risk for adverse health effects, based on studies retrieved in a broad literature search. The report concludes that glutamine is a comparatively well studied amino acid without reported changes in relevant biomarkers or organ effects in human studies. Glutamine is therefore grouped among the amino acids possessing a low potential risk for adverse health effects.

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

L-glutamine: The dose proposal evaluated by AESAN (2012) was based on the authorisation in Denmark of a total maximum amount that must not exceed 2000 mg of L-glutamine per suggested daily dose (FVM, 2011). Also, reference is made to the authorisation in Italy of the use of L-glutamine in food supplements (legislative proposal) without the establishment of a maximum daily dose (Italy, 2012). The safety evaluation cites the review paper by Garlick (2001). Limitations regarding the safety evaluation of glutamine are pointed out: i) there were no studies of any sort on the use of L-glutamine in healthy subjects over long periods of time; ii) the studies had always been conducted on patients under strict medical supervision; iii) the need to study individual susceptibility is mentioned, with reference to the study by Akobeng et al. (2000) demonstrating intolerance to glutamine in doses of 0.1-1.0 g/kg bw per day in patients with Crohn's disease; iv) there were no toxicity data available for the elderly that might represent a vulnerable group; v) L-glutamine is metabolised to glutamate and ammonia, substances that may have neurological effects, and therefore studies on possible psychological and neurological effects are required. The AESAN (2012) report cites the VKM report from 2011 which considered that L-glutamine presents a low risk as no changes occur in the biomarkers nor are there any adverse effects to health (VKM, 2011). It is stated in conclusion that no adverse effects had been observed neither in the safety studies conducted with L-glutamine nor in its use at high doses in clinical nutrition. A maximum daily amount of 2000 mg of L-glutamine was found acceptable from a safety point of view for use as a food supplement.

L-glutamic acid: The AESAN (2012) report cites studies indicating the absence of mutagenicity and effect on reproduction and embryonic development (FDA, 1969; McColl et al., 1965). Further, AESAN (2012) cites studies in mice and rats given up to 4% L-glutamic acid in the diet for one year, without any changes (Little, 1953a; Little, 1953b). Some rodent studies had indicated possible neurotoxic effects at high doses. However, it is stated that the

neurotoxic effects will not occur in humans, as the administration of doses of monosodium glutamate corresponding to palatable maximum levels in one study increased human plasma L-glutamic acid levels as little as 1/40 of that in rodents (Salmona et al., 1980). In addition, in rodents, increases of up to 10 times in the plasma level of L-glutamic acid did not induce increases in total content of L-glutamic acid in the brain, although a build-up in specific parts of the brain could not be excluded (Garattini, 2000).

It is stated in the AESAN (2012) report that it is known that L-glutamic acid is safe for the general population, with reference to a review paper by Kulkarni et al. (2005). However, also with reference to the same paper by Kulkarni, doses between 1.5-12 g of L-glutamic acid taken in a single meal after 15-25 min has been reported to produce a series of adverse effects including a burning sensation, oppression and/or numbness of the chest, which may spread to the neck, face, shoulders, and arms. Dizziness, headaches, nausea and vomiting may also occur. The underlying reference cited by Kulkarni et al. (2005) is: Truth in labelling [www.truthinlabelling.org]. The original report in this website was not found when the present report was written.

AESAN (2012) concludes that L-glutamic acid is present in protein foods in the diet, has a low level of toxicity, and adverse effects are only observed at doses above 1.5 g. A maximum amount of 1 g/day was found acceptable from a safety point of view for use in food supplements.

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 – 2. Spain, 2013

The AESAN (2013) report proposed a maximum daily quantity of 5 g of L-glutamine, based on the fact that L-glutamine had been authorised in Italy in food supplements (legislative proposal) without the establishment of a maximum daily quantity (Italy, 2012). Also cited are two opinions of the Panel on Dietetic Products, Nutrition and Allergies of the European Food Safety Authority (EFSA) (EFSA, 2009; EFSA, 2011) on the benefits claimed for glutamine. However, it should be noted that EFSA in the claims evaluation process did not evaluate safety including the doses proposed for health claims (which were, furthermore, not found to be substantiated). The AESAN (2013) report repeats the considerations regarding lack of safety information for glutamine referred above for the AESAN (2012) report, and also cites the "low risk" classification of L-glutamine in the VKM report from 2011. No studies not included in the 2012 report are referred to. However, the new proposal of a maximum daily quantity of 5 g of L-glutamine was found acceptable from a safety point of view for use as a food supplement.

2.1.2 Literature search

2.1.2.1 Search strategies

For the present report, a systematic literature search for published literature covering the period from 2000 to present was performed in MEDLINE and EMBASE in order to retrieve publications on adverse effects caused by glutamine or glutamic acid as well as glutamates. These databases were chosen to ensure comprehensive study retrieval. The literature searches were performed by the project leader on 4 June 2015.

Studies in experimental animals were identified in a separate search in the same databases 19 November 2015. And finally, a separate search was performed to possibly find additional publications on children and adolescents 2 February 2016.

The search strategies are shown in Appendix 1.

2.1.2.2 Publication selection

To retrieve any recent studies on adverse effects caused by L-glutamine and L-glutamic acid published after the search included in the latest Norwegian VKM (2011) report and the cited reports from other National Authorities, search results on human studies from 2011 to present were further evaluated for inclusion, while titles and abstracts published in the period 2000-2010 were screened to identify possible relevant studies not included in previous reports. For the search in children and adolescents, information about whether tolerance may be lower in these population groups was a special focus.

The study type for inclusion in this opinion is human studies. Included studies in animals serve as supporting evidence. The criteria for inclusion of human studies were:

- Human intervention studies with oral route of exposure to L-glutamine and L-glutamic acid or glutamates
- L-glutamine or L-glutamic acid in relation to adverse effect in humans must be addressed in the abstract or full text of the paper
- Studies performed in apparently healthy individuals or patient groups who are assumed to have normal L-glutamine and glutamic acid absorption and/or metabolism
- No co-administration of other substances that potentially might mimic or confound an effect of L-glutamine or L-glutamic acid

For studies in children and adolescents the same criteria were applied.

Studies using dipeptides e.g. alanyl-glutamine were excluded, and also studies with acetyl-glutamine.

For the animal studies search (see below), retrieved papers were included if they reported results from chronic or sub-chronic toxicity or feeding studies.

In vitro studies were not included, but were read as background information with regard to potential toxic properties of L-glutamine and L-glutamic acid.

Papers in languages other than English, Norwegian, Danish or Swedish were excluded.

The main literature search performed on 4 June 2015 identified, after removal of duplicates, 2619 publications for the period 2000-2015, of these 994 publications were from the period 2011-2015.

Study titles and abstracts for the period 2011-2015 were pre-screened by the author of this report according to the inclusion criteria listed above, together with titles reporting observational studies, studies in various patient groups and a loosely defined group of studies considered to possibly give valuable background information on mechanisms and potential toxicity. This pre-screening identified 111 papers for the period 2011-2015 to be included in the final screening by title and abstracts.

In the final screening, performed according to the inclusion criteria, 10 titles were retrieved. Seven papers retrieved concerned L-glutamine, no papers concerned glutamic acid, and three papers concerned monosodium glutamate. Additionally, one paper (glutamine) was identified by reviewing the search results 2000-2010. However, none of the papers on glutamine were studies in healthy individuals. After full text reading of these 11 articles by the author of this report and further discussion in the VKM Panel, applying the same inclusion criteria all 8 papers on glutamine were excluded. The reasons for exclusion of these papers were:

- Narrative review and adverse effects not addressed (1 paper)
- Not systematic review (1 paper)
- Not healthy individuals/ patient groups who may be suspected to have altered L-glutamine and glutamic acid absorption and/or metabolism (6 papers)

The search in children and adolescents identified no additional publications relevant for inclusion.

The search for animal studies retrieved 4 relevant reports, two of which were classical-toxicology-type studies in rats on L-glutamine and one on L-glutamic acid (n=1). One was a relevant study on protein metabolism in relation to glutamine supplementation.

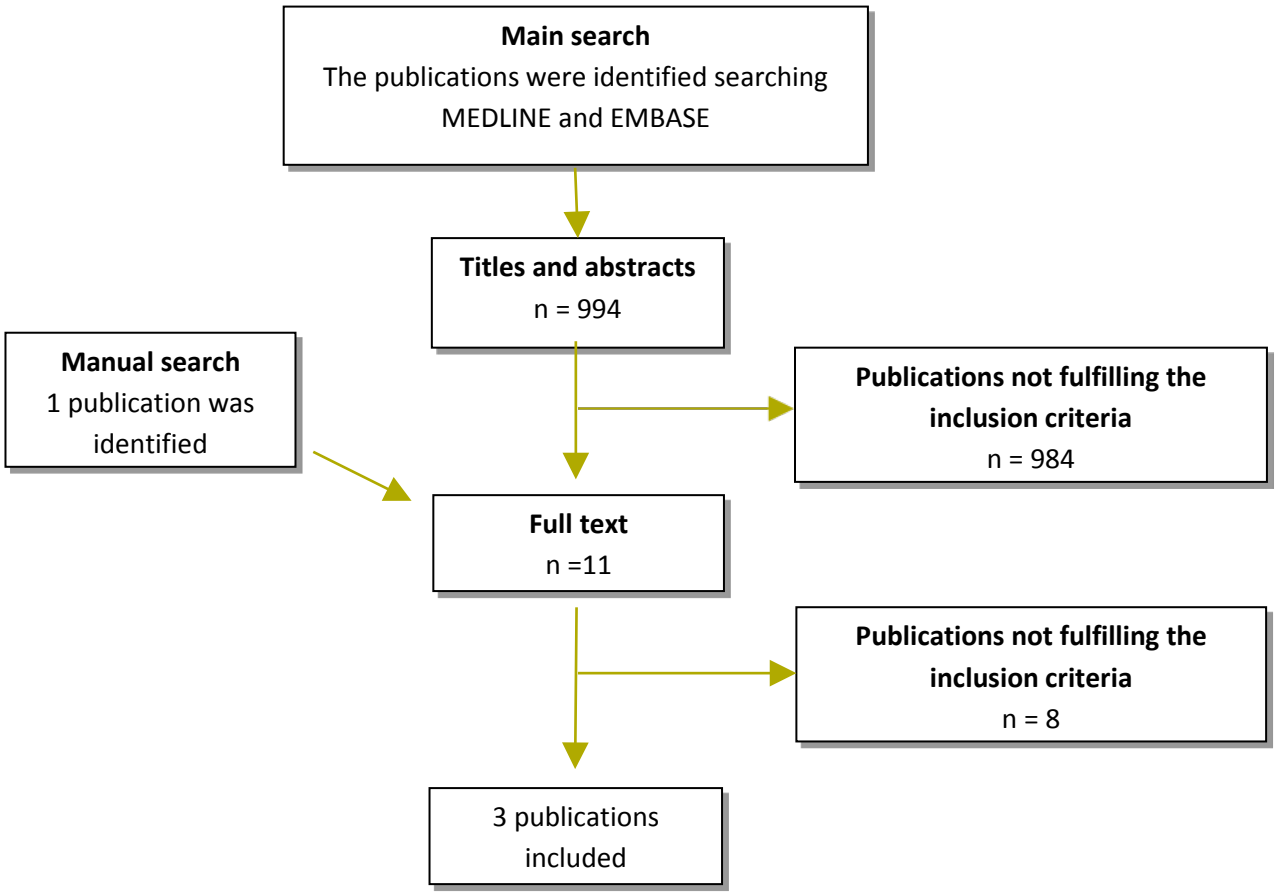
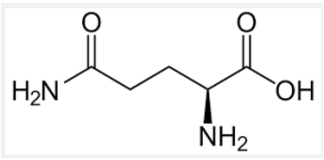


Figure 2.1.2.2-1: Flow chart for publication selection for glutamine and glutamic acid human studies literature search.

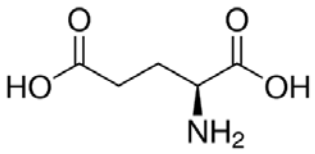
2.2 General information

2.2.1 Chemistry

L-glutamine (CAS number 56-85-9), chemical formula $C_5H_{10}N_2O_3$ is an amide of glutamic acid (see structural formulas, Fig. 2.2.1-1) with molecular weight 146.14. It contains an α-amino group, an α-carboxylic acid group and a side chain amide which replaces the side chain hydroxyl of glutamic acid. Glutamine is a non-essential amino acid in the human body (considered conditionally essential in states of disease and stress). Glutamine is synthesised by the enzyme glutamine synthetase from glutamic acid and ammonia, and is hydrolysed by glutaminase to glutamic acid and ammonia. Glutamine is the most abundant amino acid in human blood. This assessment only deals with L-glutamine, which is the predominant form of glutamine naturally occurring in the body and used in supplements. Less is known about the function and metabolism of the D-form of glutamine.



Glutamine



Glutamic acid

Figure 2.2.1-1: Structural formulas for glutamine and glutamic acid.

L-glutamic acid (CAS number 56-86-0), chemical formula $C_5H_9NO_4$ is a non-essential amino acid with molecular weight 147.13. At physiological conditions (physiological pH) its side chain is fully ionised, i.e. L-glutamic acid exists in the form of glutamate with a negatively charged carboxylate group (COO^-). Removal of the amino group from glutamate by the enzyme glutamate dehydrogenase gives α-ketoglutarate which feeds into the tricarboxylic acid cycle. This assessment only deals with L-glutamic acid, which is the predominant form of glutamic acid naturally occurring in the body and used in supplements. Less is known about the function and metabolism of the D-form of glutamic acid.

2.2.2 Occurrence

Glutamine is the most abundant naturally occurring amino acid in the human body. In the body, it is stored mainly in the skeletal muscle mass.

L-glutamine is available from all protein-containing foods. High-protein foods contain the most (e.g. meat, fish, eggs and dairy products). Similarly, all meats, poultry, fish, eggs, and dairy products are excellent sources of glutamic acid. Some protein-rich plant foods also serve as sources. About 30% to 35% of the protein in wheat is glutamic acid.

2.3 Absorption, distribution, metabolism and excretion

Absorption and distribution

Glutamine and glutamic acid/glutamate are absorbed from the intestine, but are also to a large extent synthesised by the body. Glutamine is taken up by the intestinal epithelium by means of amino acid transporters, to be converted to glutamate and α-ketoglutarate for the production of alanine. Alanine will mainly serve as a substrate for gluconeogenesis and energy production.

On the general level, glutamine has several cell membrane transporters. Concentrative transporters allow glutamine absorption from diet in intestine and reabsorption from urine in kidney. On the other hand, both co-transporters and antiporters allow delivery of glutamine in all the tissues and equilibration with other amino acids (Pochini et al., 2014). Transport of different amino acids can be interrelated, e.g. transporter SLC1A5 regulates an increase in the intracellular concentration of L-glutamine, and transporter SLC7A5/SLC3A2 uses intracellular L-glutamine as an efflux substrate to regulate cellular uptake of extracellular L-

leucine (Nicklin et al., 2009). Glutamine was found to inhibit uptake of serine and alanine by liver cells (Joseph et al., 1978). The redundancy of glutamine transporters points to their importance in homeostasis; however, much remains to be described regarding glutamine transporters and interactions with other amino acids.

Glutamine and glutamic acid/glutamate are important in carbohydrate- and energy metabolism (via the citric acid cycle), protein metabolism and nitrogen handling (via urea cycle), as building blocks for a number of molecules e.g. purines, as neurotransmitters and signal substances, and generally take part in many complex metabolic processes. Glutamine is required for the uptake of essential amino acids and as a nitrogen donor in as many as three independent enzymatic steps for purine synthesis and in two independent enzymatic steps for pyrimidine synthesis forming essential bases for deoxyribonucleic acid (DNA) production. Glutamine participates in other metabolic pathways e.g. the enzymes involved in nucleotide biosynthesis contribute to the conversion of glutamine to glutamic acid. The enzyme glutaminase also contributes to this activity through the release of glutamine's amide group as free ammonia. The generated glutamic acid can be converted into α-ketoglutarate through the cellular transaminases or through glutamic acid dehydrogenase, which catalyses $(NAD(P)^+)$-production. NADPH in addition to being vital to mitochondrial red/ox potential is essential to biosynthesis of macromolecules.

Endogen production of glutamine is estimated to 60 -100 mg/day (van Acker et al., 1999) and several investigators have reported that plasma glutamine levels did not rise significantly during glutamine supplementation (Benjamin et al., 2012; Mansour et al., 2015; van Acker et al., 2000a; van Acker et al., 2000b; Wilmore, 2001). This indicates that glutamine supplementation with the doses may (10 g x 3 per day; 0.5 g/kg ideal body weight/day perorally), may not significantly increase plasma glutamine levels in individuals with an already normal plasma level, apparently due to intestinal, liver and muscle cell uptake. nicotinamide adenine dinucleotide phosphate Regarding glutamic acid, ninety-five percent of the dietary glutamate is metabolised to CO_2 (50%) and various amino acids by intestinal cells in a first pass (Reeds et al., 2000).

The most relevant glutamine-producing tissue in the body is the muscle mass, accounting for about 90% of all glutamine synthesised. An important source is the catabolism of branched-chain amino acids. The role of the liver in glutamine metabolism is more regulatory than producing.

Glutamine is found in plasma at concentrations higher than other amino acids, consistent with its transport function (see below).

High-consumers of glutamine include the cells of intestine, the kidney cells for the acid-base balance, and activated immune cells. Glutamine is also taken up by cells of the liver and muscle.

Glutamine is one of the few amino acids that can directly cross the blood-brain barrier. In the CNS, L-glutamine is essential for production of the transmitter substance L-glutamate (see below) as L-glutamate is excluded from the CNS by the blood-brain barrier.

Regarding extrapolation from animals to humans, we are not aware of any studies demonstrating differences in glutamine metabolism of significance for risk evaluation between rodents and humans.

Metabolism, physiological function and excretion

Glutamine and glutamic acid play important roles in many metabolic processes:

- Protein synthesis, as any other of the proteogenic amino acids
- Nitrogen donation for other anabolic processes
- Regulation of acid-base balance in the kidney (by producing ammonium)
- Glutamine synthesis is important in the brain, where it is the major mechanism for removal of ammonia. Glutamine synthetase acts as a general ammonia 'scavenger'.
- Ammonium handling in general via the urea cycle
- Nontoxic transporter of ammonia in the blood circulation
- Carbon donation, as a source feeding into the tricarboxylic acid cycle via α-ketoglutarate
- Cellular energy, as a source feeding into the citric acid cycle
- In pancreatic beta-cells, oxidation of glutamate mediates amino acid-stimulated insulin secretion.
- Neurotransmission

Glutamic acid (in the form of glutamate) is the most common excitatory neurotransmitter in the CNS, and additionally is a precursor for the synthesis of GABA in inhibitory GABAergic neurons. Changes in glutamic acid/glutamate and GABA metabolism may play important roles in the control of brain cortical excitability. L-glutamate levels of the neuronal cytoplasm are 10 000 times greater than the extracellular fluid. This remarkable stratification of brain glutamate levels is accomplished primarily via the glutamate–glutamine cycle which is essential to brain glutamate metabolism. In the glutamate–glutamine cycle, L-glutamate is released from nerve endings into the synapse, where it has an excitatory effect. Astrocytes rapidly take up the released L-glutamate, thereby maintaining a low intra-synaptic level. Within astrocytes, L-glutamate is converted to L-glutamine in the presence of circulating ammonia. L-glutamine is release via a specialised transport to adjacent neurons where it is converted to L-glutamate this way supporting a steady supply of precursor for the synthesis of L-glutamate. Exclusion of L-glutamate from entering the CNS is in part the reason why L-glutamine can be supplied through foods in large quantities without having adverse effects on CNS function (Smith, 2000).

Disruptions of glutamate metabolism have been implicated in a number of clinical disorders, such as certain types of hyperinsulinism and seizures (Kelly and Stanley, 2001).

Amino acids in the healthy individual are not excreted, but only disposed off after metabolism as urea.

2.4 Toxicological data / Adverse effects

2.4.1 Human studies

Monosodium glutamate avoidance for chronic asthma in adults and children. Zhou et al., 2012.

The objectives of this Cochrane review was to identify controlled randomised trials (RCTs) of monosodium glutamate intake and asthma response in adults and children older than two years with chronic asthma, to assess the methodological quality of the publications, and determine the effects of monosodium glutamate on asthma outcomes – maximum fall in forced expiratory volume in the first second (FEV1) greater than 15% or 200 ml after monosodium glutamate or the control challenge (Zhou et al., 2012). Two studies published in 1987 and 1998, respectively, could be included; 34 retrieved publications were excluded. In addition to the main outcome, data on symptom scores, non-specific bronchial hyper-responsiveness (BHR), eosinophil cationic protein (ECP) and tryptase levels were provided. Number of participants was 24 adults, no children. No evidence that monosodium glutamate worsens asthma was found, but the limitations of the review including the low number of study subjects are pointed out.

Headache and mechanical sensitization of human pericranial muscles after repeated intake of monosodium glutamate (MSG). Shimada et al., 2013

Fourteen healthy subjects participated in 5 daily sessions for one week of MSG intake (150 mg/kg equalling 10.5 g for a 70 kg person) or placebo (24 mg/kg NaCl) (randomised, double-blinded) (Shimada et al., 2013). Spontaneous pain, pressure pain thresholds and tolerance levels for the masseter and temporalis muscles, side effects, and blood pressure were evaluated before and 15, 30, and 50 min after MSG intake. Headache occurred in 8/14 subjects during MSG and 2/14 during placebo ($p= 0.041$). Blood pressure was significantly elevated after MSG ($p<0.040$). The results are compatible with the notion that high doses of glutamate can trigger symptoms related to the autonomic nerve system. However, blinding was incomplete, as subjects correctly identified the substance given to them in 88% of the occasions, suggesting that most subjects were able to guess what they were receiving. For this reason and the low number of study subjects, the strength of evidence provided by this study is low and the study inconclusive.

Differential effects of repetitive oral administration of monosodium glutamate on interstitial glutamate concentration and muscle pain sensitivity. Shimada et al., 2015

This randomised, double-blinded, placebo-controlled study was performed to determine the relationship of high-dose daily MSG consumption with glutamate concentrations in jaw

muscle, saliva, and serum, and muscle pain sensitivity in healthy participants (Shimada et al., 2015). In five contiguous experimental daily sessions, 32 healthy participants drank MSG (150 mg/kg, equalling 10.5 g for a 70 kg person) or NaCl (24 mg/kg) diluted with 400 mL aspartame-sweetened soda. Subjective adverse effects were recorded (nine categories: nausea, headache, dizziness, chest pressure/tightness, burning sensation, fatigue, soreness in jaw/jaw muscles, abdominal pain, others), as well as systolic and diastolic blood pressure and heart rate. Subjective adverse effects were recorded in 22 cases in the glutamate group and 10 cases in the placebo group, with a statistically significantly higher frequency in the glutamate group for nausea (9 vs 0, p=0.03) and headache (11 vs 1, p=0.049). Also, statistically significant increases in systolic and diastolic blood pressures after MSG administration were observed. Completeness of blinding was not evaluated, but similar to a previous study (Shimada et al., 2013), sugar-free soda was used as the medium for glutamate administration as well as for placebo. In the previous study incomplete blinding was reported (see above). The results of the present study are in line with the notion that high doses of glutamate can trigger symptoms related to the autonomic nerve system, but uncertainty about the completeness of blinding reduces the strength of the evidence provided by this study which is found to be inconclusive.

2.4.2 Animal studies

In the animal studies search, two relevant studies were identified from 2011 or later, and it was found appropriate to additionally include two earlier studies, one of which (Harper et al., 2009) had not been cited in previous reports. Thus, a total of four animal studies were included in the present report (Table 2.4.2-1).

Table 2.4.2-1: Overview of included studies investigating L-glutamate or L-glutamic acid in relation to adverse health effects, identified in the systematic literature search for animal studies.

Reference	Animals	Sub-stance	Doses	Main endpoints	Duration of exposure	Adverse effects	NOAEL[1] (mg/kg bw/day)
Holecek (2011)	Wistar rats, male 7 to 10/group, male	Gluta-mine	Standard diet, glutamine supple-mented diet (49% of nitro-genous substan-ces), high protein diet (iso-nitro-genous control)	Protein metabolism, amino acids in body fluids	3 months dietary study, final 24 hour fasting	Changes in several amino acid concentrations, negative effect on protein balance in skeletal muscle, impaired response to starvation	n.a.[2]
Wong et al. (2011)	Sprague-Dawley rats 10/sex/group	L-glutamin e	0, 0.5, 2.5, 5% (top 4515 mg/kg bw/day) in feed	OECD Guide-line 407-like tox study, incl genotoxicity	13-week dietary study	None	M: 3832 F: 4515
Harper et al. (2009)	Sprague-Dawley rats 10/sex/group	L-glutamic acid	1000 mg/kg bw/day Target: 1000 mg/kg bw day	OECD Guide-line 407	28-day dietary study	None	M: 953 F: 1047
Tsubuku et al. (2004)	Sprague-Dawley rats 12/sex/group	L-gluta-mine	0, 0.5, 2.5, 5 % (top 4515 mg/kg bw/day) in feed	Standard tox study, incl genotoxicity	13-week dietary study + 5 week recovery	Urine total protein, pH, ketone bodies Blood platelets, γ-globulin, lactate dehydrogenase (within normal range)	M: 830 (3832)[3] F: 960 (4515)[3]

[1]except for the Tsubuku paper, all values represent the highest dose tested.
[2]n.a. = not addressed.
[3]the lower values set by the authors; alternative evaluation of results (see Wong et al., 2011) in parenthesis.

Adverse effects of chronic intake of glutamine-supplemented diet on amino acid concentrations and protein metabolism in rat: Effect of short-term starvation. Holecek, 2011

The purpose of this study was to investigate whether chronic intake of a glutamine-supplemented diet would affect concentrations of free amino acids and protein turnover in selected tissues in the white rat (Holecek, 2011). A further aim was to evaluate how chronic intake of high amounts of glutamine affects the response to short-term starvation. Male Wistar rats were randomised to three groups fed either standard laboratory diet, glutamine-supplemented diet (standard laboratory diet:glutamine 5:1) or an isonitrogenous high protein diet (standard laboratory diet:casein 5:1). In the glutamine-supplemented diet, glutamine accounted for 49% of nitrogenous substances, according to the author resembling the situation in some high-dose humane studies on glutamine supplementation. After three

months, fed and 24-hour fasted animals were examined. There was no diet-related difference in food intake or weight gain. In the animals sacrificed in the fed state, blood plasma of glutamine-supplemented animals had higher concentrations of glutamine, histidine and urea, and lower concentrations of a number of amino acids as compared to plasma from control animals (µmol/l (urea mmol/l); means ± SE; ctr vs suppl; $p<0.05$: urea 8.6 ± 0.4 vs 13.5 ± 0.3; glutamine 781 ± 23 vs 1023 ± 38; histidine 77 ± 2 vs 101 ± 4; glutaminsyre 100 ± 6 vs 92 ± 4); serine 294 ± 11 vs 220 ± 5; glycine 325 ± 18 vs 137 ± 8; threonine 304 ± 24 vs 194 ± 7; arginine 188 ± 4 vs 159 ± 4; proline 270 ± 15 vs 220 ± 9; cysteine 131 ± 7 vs 113 ± 2; valine 215 ± 9 vs 183 ± 5; isoleucine 91 ± 5 vs 74 ± 2; leucine 148 ± 9 vs 123 ± 4; lysine 348 ± 11 vs 248 ± 6; BCAA 454 ± 23 vs 379 ± 11). Main alterations in intracellular free amino acid concentrations in supplemented animals were increased levels of glutamine, increased concentrations of histidine in skeletal muscle, and decreased concentrations of glycine, serine, and threonine in several tissues. Amino acid changes differed between rats fed glutamine-supplemented diet and rats fed high-protein diet. Further, in animals fed a supplemented diet, there was an enhanced amount and concentration of protein in the kidneys, decreased protein content in in tibialis muscle, and enhanced chymotrypsin-like activity (measure of proteolysis) in soleus muscle. An explanation for the observed amino acid changes may be competitive and non-competitive inhibition by glutamine of the specific transporters across cell membranes for various amino acids. No significant effect on protein synthesis was found in any tissue. Starvation in rats previously fed a supplemented diet led to a marked decrease in glutamine concentration and an increase in glycine, serine, and threonine in plasma, and in a significant decrease in protein synthesis in the liver, jejunum, colon and spleen, all compared to animals fed the standard diet before starvation. A similar effect on protein synthesis was also observed in animals fed a high-protein diet. The author concluded that chronic intake of the glutamine-supplemented diet significantly altered amino acid concentrations in plasma and tissues, and resulted in an impaired response to starvation, in particular impaired protein synthesis in visceral tissues. The author advised against high-dose glutamine supplementation, in particular in the absence of a demonstrated glutamine deficit.

Oral subchronic and genotoxicity studies conducted with the amino acid, l-glutamine. Wong et al., 2011

To assess the safety of L-glutamine, the authors performed studies to investigate possible genotoxic and mutagenic effects of L-glutamine, a 14-day dose-finding study, and a 13-week repeated dose toxicity study (Wong et al., 2011). Five-week-old Sprague-Dawley rats, randomised into groups of 10 animals of each sex and housed individually, were used. L-glutamine did not display mutagenic or genotoxic properties, and in the 13-week study with doses of 0 (control), 0.5, 1.0 and 5% of L-glutamine no mortality, clinical signs, behavioural changes or ophthalmological abnormalities were observed. No significant differences in food consumption, body weight gain or body weight, urine analysis parameters, haematology parameters, macroscopic or histopathological organ findings or absolute and relative organ weights were detected. A number of small differences between some experimental groups in clinical chemistry after administration of glutamine were judged not to be toxicologically

relevant as they were not dose-dependent and were within the range of the laboratory's historical data. A NOAEL of 5% of glutamine in the diet was established, corresponding to the highest concentration tested, equal to 3832 mg/kg bw per day for male rats and 4515 mg/kg bw per day for female rats.

N-acetyl-glutamic acid: Evaluation of acute and 28-day repeated dose oral toxicity and genotoxicity. Harper et al., 2009

An acute and 28-day repeated dose oral toxicity and genotoxicity study of N-acetyl glutamic acid and L-glutamic acid was performed in ICR mice (mutation and micronucleus assays) and in Sprague-Dawley rats of both sexes according to OECD guidelines and good laboratory practice (GLP) (Harper et al., 2009). No evidence of genotoxicity was observed, neither was there any sign of acute toxicity after administration of N-acetyl glutamic acid or L-glutamic acid at a dose of 2000 mg/kg. In the repeated-dose 28-day study, N-acetyl glutamic acid or L-glutamic acid was given in the diet at doses of 90.9, 443.6, and 914.2 mg/kg bw per day (males) and 106.2, 497, and 1006.6 mg/kg bw per day (females); additional groups consumed L-glutamic acid 953.0 mg/kg bw per day (males) or 1046.7 mg/kg bw per day (females). No adverse effects were observed in relation to body weight, clinical signs, serum chemistry, haematology and coagulation values, or macroscopic or microscopic pathology. A small, statistically significant increase was observed in blood leukocyte (32% increase) and lymphocyte (41% increase) counts in high dose males, and was also observed with L-glutamic acid; however, the values were within historical control limits in the lab and were not considered to be adverse. It was concluded that the highest dose tested for glutamic acid, 953 mg/kg bw per day for males and 1047 mg/kg bw per day for females represented the no observed adverse effect level (NOAEL).

Thirteen-week oral toxicity study of L-glutamine in rats. Tsubuku et al., 2004

Male and female individually housed Sprague-Dawley rats were used in this GLP study (Tsubuku et al., 2004). The study appears adequately performed and well described. After a two-week acclimatisation period, four groups of 12 rats (age 6 weeks) (control group and three doses of glutamine) were selected out of 75 animals by a computer program to ensure homogenous weight. For a 13-week period the rats were fed either glutamine incorporated into a standard diet at doses equal to 1.25%, 2.5%, and 5.0% (w/w), or only the standard diet. Diet intake was measured twice a week, and glutamine intake calculated. Ophthalmologic examination and a number of urine parameters were examined, and at the end of the administration period and a 2-week recovery period haematology and blood chemistry, bone marrow, macro pathology and histopathology of a large number of organs. Glutamine doses ranged from 833±14 mg/kg bw per day (mean±SD; 1.25% glutamine diet) to 3379±119 mg/kg per day (5% glutamine diet) in males and 964±55 mg/kg per day to 4026±216 mg/kg per day in females. No deaths or clinical signs and no glutamine-related changes in weight, water intake or ophthalmological abnormalities were observed. Several changes in urine parameters were observed in the 2.5% and 5% glutamine groups (total protein, pH, ketone bodies) at the end of the administration period, and in the 5% group

also minor changes (all within the physiological range) in platelet count, γ-globulin and lactate dehydrogenase. No effects of glutamine administration were observed in the 1.25% glutamine group. The NOAEL for glutamine was estimated to be 1.25% for both genders (males 0.83±0.01 g/kg per day; females 0.96±0.06 g/kg per day). The conclusion on the NOAEL by the authors have been disputed by other investigators, arguing that the effects observed were not toxicologically relevant, and that the NOAEL observed in this study should instead be the highest dose used, equal to 3832 mg/kg bw per day glutamine for males and 4515 mg/kg bw per day glutamine for females (Wong et al., 2011). VKM is of the same opinion.

2.4.3 Mode of action for adverse effects

Adverse effects reported by some subjects during L-glutamine supplementation, specifically loose stools, may possibly be due to an osmotic effect. Headache experienced by some subjects has not been attributed to specific mechanisms.

With regard to L-glutamic acid, no reports of specific adverse effects were retrieved. Regarding glutamate, no specific mechanism has been proposed for the putative adverse effects reported.

2.4.4 Vulnerable groups

Children and adolescents: Any evidence to set a tolerance level for L-glutamine and L-glutamic acid specifically for children or adolescents has not been found. Therefore, an assumption is made that these age groups have similar tolerance as adults relative to their body weight.

Fetuses, pregnant and lactating women: It is not known whether moderate supplementation with L-glutamine or L-glutamic acid has any effect on the human fetus, or whether tolerance is different in pregnant and lactating women. It should be noted that pregnant and lactating women may constitute a health-conscious group prone to ingest dietary supplements.

The elderly: A two-week study reported by Galera et al. (2010) was in institutionalised elderly subjects with various ailments (mean age ± SD was 69± 8.8. years) who consumed glutamine 0.5 g/kg bw per day or calcium caseinate. There was no control group. Blood urea levels increased in both groups, presumably due to the increased nitrogen intake. Also in studies by Samocha-Bonet et al. (2014) and Mansour et al. (2015) in diabetic individuals blood urea levels increased. However, only in the Galera et al. (2010) study was there also a rise in serum creatinine concentrations and reduced estimated glomerular filtration rate (both in the glutamine and the casein groups), possibly due to the generally reduced renal function capacity in the elderly.

Extracellular fluid volume expansion was indicated by laboratory parameters in the 14-day study of high-dose glutamine and casein supplementation in elderly institutionalised volunteers Galera et al. (2010), an observation made also in a study in otherwise healthy diabetic subjects by Samocha-Bonet et al. (2014) and in a study of glutamine supplementation to piglets (House et al., 1994). The long-term physiological importance of this finding remains to be clarified.

Galera et al. (2010) did not observe adverse clinical effects or clinically significant laboratory changes in their 2-week study in institutionalised elderly subjects, but the findings referred indicate that renal function and electrolyte balance should be supervised in elderly individuals taking high-dose glutamine supplementation.

Impaired renal or hepatic function: As discussed above, high-dose glutamine supplementation in elderly subjects has been reported to be associated with increased serum creatinine values (Galera et al., 2010). Individuals with impaired renal or hepatic function taking glutamine supplementation may be at risk for adverse effects and should be supervised with regard to renal function, hyperammonemia and associated parameters.

Diabetes: Samocha-Bonet et al. (2011) and Samocha-Bonet et al. (2014) found that glutamine supplementation was associated with a reduced postprandial glycemic response, and enhanced insulin and GLP-1 responses (glucagon-like peptide-1). The effect on blood glucose is in line with previous reports that glutamine stimulates the secretion of GLP-1 and insulin in obese and type-2 diabetic subjects (Greenfield et al., 2009; Reimann et al., 2004). Although these effects are not adverse in the short term, the findings indicate a need for further safety studies in diabetic subjects

Mental disorders: No direct scientific evidence that the intake levels of glutamine or glutamic acid affects mental function has been retrieved.

2.4.4.1 Interactions, allergic sensitisation and adjuvant effects

An increase in absolute or relative lymphocyte counts has been noted in several studies on glutamine supplementation (Galera et al., 2010; Garlick, 2004; Heyland et al., 2006; Roth, 2007; Roth et al., 2002); however, the consequences for immune function, if any, are not known. From animal experiments, there is some evidence that the level of glutamine can affect the expression of e.g. regulatory T cell populations and thereby affect inflammation, immune defense and allergy (Metzler et al., 2016).

It has been reported that dietary L-glutamine supplementation modulates the microbial community and activates innate immunity in the mouse intestine (Ren et al., 2014). The situation in humans and possible long-term consequences remain to be clarified.

Concerning allergic sensitisation or allergy adjuvant effects, there was no information in the literature reviewed in the present risk assessment. The scarce information in the selected literature does not document an absence of allergic sensitisation or allergy adjuvant effects.

2.5 Summary of hazard identification and characterisation

For L-glutamine new human studies reporting on adverse events (or the absence of such events) in healthy individuals were not retrieved, and long-term studies in humans are still essentially missing. Information on metabolic and adverse effects observed in studies in humans with various disease conditions can only serve as background information. Specific information about potential negative health effects and the associated doses can be derived from the information retrieved in the literature search for animal studies, and evaluated against the background information summarised in previous safety assessment reports. A 'classical' toxicology study according to OECD Guideline 407 not cited in previous reports (Wong et al., 2011) was included. The NOAELs were derived from the highest dose of L-glutamine tested in these studies. Data from studies in various patient groups (previous reports (IOM, 2005; VKM, 2011);(Benjamin et al., 2012; Galera et al., 2010; Guo et al., 2013; Mansour et al., 2015; Samocha-Bonet et al., 2014) support the data from the two animal studies indicating the absence of significant adverse effects with doses in the range proposed.

For L-glutamic acid, similarly no new human studies have been found apart from three studies on glutamate. A 'classical' toxicology study according to OECD guideline 407 not cited in previous reports (Harper et al., 2009) was included. The NOAEL was derived from the only dose of L-glutamic acid tested in this study, which did not reveal any adverse effects. Data from early long-term studies in animals and humans as well as short-term studies on glutamates cited in previous reports support the data from the animal study indicating the absence of significant adverse effects with doses in the range proposed.

However, the question of what uncertainty factor is appropriate in OECD Guideline 407-studies with a substance that is a major nutrient is difficult. Although an uncertainty factor of 100 ideally should be used also in relation to feed studies, this will sometimes give values lower than the lowest daily requirements for the nutrient in question, and in particular when the NOAEL is derived pragmatically from the highest (and sometimes only) dose tested (ANSES, 2011).

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

Glutamine:

1. A NOAEL of 3832 mg/kg bw per day (highest dose tested) for glutamine has been identified in two 90-day toxicological studies in rats
2. No long-term studies on L-glutamine in healthy adult humans were found, and no relevant studies have been identified in children or adolescents
3. In short-term human studies in different patient groups, glutamine has been well tolerated, with the only observed negative health effects being loose stools, general abdominal discomfort and headache.
4. Safety evaluation of glutamine as supplement must at present be based largely on animal studies, together with current knowledge of short-term glutamine metabolism

and information from studies in subjects with various diseases. Only the animal studies allow evaluation of specific doses.

Glutamic acid

5. A NOAEL for L-glutamic acid of 953 mg/kg bw per day (only dose tested) has been identified in a toxicological 28-day study in rats.
6. No long-term studies on L-glutamic acid in healthy adult humans were found, and no relevant studies have been identified in children or adolescents
7. Adverse effects from L-glutamic acid in doses over 1500 mg/day in humans have been claimed, however it has not been possible to retrieve the basis for this claim that appears to have been posted on a website (AESAN, 2012)
8. Adverse effects from monosodium glutamate ingestion at doses of 2500 mg/day and 5000 mg/day have also been claimed; however the findings were inconsistent and not reproducible (IOM, 2005)

For the risk characterisation of L-glutamine, in the absence of long-term human studies in healthy individuals, VKM will base the value of comparison on the highest dose tested (NOAEL) in two 90-day studies in rodents. Employing an uncertainty factor of 10 for the extrapolation between species, the value of comparison is set to 383.2 mg/kg bw per day, corresponding to 26.8 g per day in a 70 kg adult.

In the risk characterisation of L-glutamic acid, in the absence of reproducible adverse effect in short-term human studies and an absence of long-term studies in healthy individuals, VKM will base the value of comparison on the highest dose tested (NOAEL) in a 28-day study in rodents. Employing an uncertainty factor of 10 for the extrapolation between species, the value of comparison is set to 95.3 mg/kg bw per day corresponding to 6.7 g/day in a 70 kg adult.

3 Exposure

Exposures of L-glutamine and L-glutamic acid were estimated from the intake of food supplements for the age groups 10-14 years, 14-18 years and adults (≥18 years).

3.1 Food supplements

The NFSA has requested a risk assessment of the doses 3500, 5000, 8000, 10000, 12000, 15000 and 16500 mg/day of L-glutamine in food supplements. For L-glutamic acid in food supplements the doses were 1000, 2000, 3000, 4000, 5000 and 5500 mg/day. The default body weights (bw) for the relevant consumer 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 is given in (Table 3.1-1)

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

Groups	Daily doses, L-glutamine, mg	Body weight	Exposures (mg/kg bw per day)
Children (10 to <14years)	3500, 5000, 8000, 10000, 12000, 15000, 16500	43.4	81, 115, 184, 233, 277, 346, 380
Adolescent (14 to <18 years)	3500, 5000, 8000, 10000, 12000, 15000, 16500	61.3	57, 82, 131, 163, 196, 245, 269
Adults (≥18 years)	3500, 5000, 8000, 10000, 12000, 15000, 16500	70.0	50, 71, 114, 143, 171, 214, 236

Table 3.1-2. Estimated exposure of L-glutamic acid in children, adolescents and adults from specified doses in food supplements.

Groups	Daily doses, L-glutamine, mg	Body weight	Exposures (mg/kg bw per day)
Children (10 to <14years)	1000, 2000, 3000, 4000, 5000, 5500	43.4	23, 46, 69, 92, 115, 127
Adolescent (14 to <18 years)	1000, 2000, 3000, 4000, 5000, 5500	61.3	16, 33, 49, 65, 82, 90
Adults (≥18 years)	1000, 2000, 3000, 4000, 5000, 5500	70.0	14, 29, 43, 57, 71, 79

Exposures of L-glutamine and L-glutamic acid were estimated from the intake of food supplements alone.

3.2 Other sources

There are no data available concerning dietary intake of L-glutamine and L-glutamic acid in Norway. Estimated from NHANES III, mean glutamic acid intake for the US adult population was approximately 15 g/day, while men 31 through 50 years had the highest reported intake at the 99th percentile of 33.7 g/day (IOM, 2005). We have found no available data on dietary intakes of L-glutamine.

4 Risk characterisation

L-glutamine

The doses of L-glutamine received from NFSA in food supplements are 3500, 5000, 8000, 10000, 12000, 15000 and 16500 mg/day. The estimated exposures for adults, adolescents and children above 10 years based on these doses are given in chapter 3.

The value of comparison is set to 383.2 mg/kg bw per day, corresponding to 26.8 g per day in a 70 kg adult. This is based on the toxicological animal studies, employing an uncertainty factor of 10 for the extrapolation between species.

No studies with glutamine in children were found. However, there are no data known indicating that children and adolescent are more vulnerable than adults for glutamine. No tolerance level is set for glutamine specifically for children or adolescents. Assuming similar tolerance for these age groups as for adults, the same value for comparison as for adults are used for children and adolescents (383.2 mg/kg bw per day).

VKM considers that:

In adults (≥18 years), the specified doses of 3500, 5000, 8000, 10000, 12000, 15000 and 16500 mg/day mg/day L-glutamine in food supplements are considered unlikely to cause adverse health effects.

In adolescents (14 to <18 years), the specified doses of 3500, 5000, 8000, 10000, 12000, 15000 and 16500 mg/day mg/day L-glutamine in food supplements are considered unlikely to cause adverse health effects.

In children (10 to <14 years), the specified doses of 3500, 5000, 8000, 10000, 12000, 15000 and 16500 mg/day mg/day L-glutamine in food supplements are considered unlikely to cause adverse health effects.

L-glutamic acid

The doses of L-glutamic acid received from NFSA in food supplements are 1000, 2000, 3000, 4000, 5000 and 5500 mg/day. The estimated exposures for adults, adolescents and children above 10 years based on these doses are given in chapter 3.

The value of comparison is set to 95.3 mg/kg bw per day corresponding to 6.7 g/day in a 70 kg adult. This is based on the toxicological 28-day animal study, employing an uncertainty factor of 10 for the extrapolation between species.

No studies with glutamic acid in children were found. However, there are little data indicating that children and adolescent are more vulnerable than adults for glutamic acid. No tolerance level is set for glutamic acid specifically for children or adolescents. Assuming similar

tolerance for these age groups as for adults, the same value for comparison as for adults are used for children and adolescents (95.3 mg/kg bw per day).

VKM considers that:

In adults (≥18 years), the specified doses of 1000, 2000, 3000, 4000, 5000 and 5500 mg/day L-glutamic acid in food supplements are considered unlikely to cause adverse health effects.

In adolescents (14 to <18 years), the specified doses of 1000, 2000, 3000, 4000, 5000 and 5500 mg/day L-glutamic acid in food supplements are considered unlikely to cause adverse health effects.

In children (10 to <14 years), the specified doses 1000, 2000, 3000, and 4000 mg/day L-glutamic acid in food supplements are considered unlikely to cause adverse health effects. The specified doses of 5000 and 5500 mg/day may represent a risk of adverse health effects.

5 Uncertainties

- A major uncertainty for the conclusion is the lack of studies in healthy adults, children and adolescents reporting on potential adverse health effects of L-glutamine and L-glutamic acid supplementation
- Similarly, data on long-term metabolic effects (e.g. diabetes) and long-term effects on e.g. central nervous function and immune system function are not available
- Uncertainty about relevant safety factors for extrapolation to humans from toxicological studies on major nutrients in rodents
- The risk assessment is based on default body weights determined by EFSA. With use of the default average body weight of an age (population) group, the variance in all individuals in the group will not be covered. Individuals with body weights less than the default estimate in a given age group are not fully covered in the risk estimate

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-glutamine and L-glutamic acid in food supplements at the following doses: 3500 mg/day, 5000 mg/day, 8000 mg/day, 10000 mg/day, 12000 mg/day, 15000 mg/day, and 16500 mg/day, and for L-glutamic acid in food supplements: 1000 mg/day, 2000 mg/day, 3000 mg/day, 4000 mg/day, 5000 mg/day, and 5500 mg/day for the general population, ages 10 years and above.

For L-glutamine specific information about potential negative health effects and the associated doses has mainly been derived from the information retrieved in the updated literature search, and has been evaluated against the background information summarised in previous safety assessment reports.

In the risk characterisation of L-glutamine, VKM used as value for comparison 383.2 mg/kg bw/day corresponding to 26.8 g per day in a 70 kg adult. This value is based on the toxicological animal studies. An uncertainty factor of 10 for inter-species extrapolation was employed.

For L-glutamic acid, VKM used as value for comparison 95.3 mg/kg bw per day corresponding to 6.67 g per day in a 70 kg adult. This value is based on a toxicological 28-day animal study. The uncertainty factor for extrapolation from rodents to humans was set to 10.

No evidence was found to assume specific tolerance levels for L-glutamine or L-glutamic acid for children or adolescents. Therefore, a similar tolerance as for adults relative to body weight was assumed for these age groups.

VKM concludes that:

L-glutamine

- In adults (≥18 years), the specified doses of 3500, 5000, 8000, 10000, 12000, 15000 and 16500 mg/day mg/day L-glutamine in food supplements are considered unlikely to cause adverse health effects.
- In adolescents (14 to <18 years), the specified doses of 3500, 5000, 8000, 10000, 12000, 15000 and 16500 mg/day mg/day L-glutamine in food supplements are considered unlikely to cause adverse health effects.

- In children (10 to <14 years), the specified doses of 3500, 5000, 8000, 10000, 12000, 15000 and 16500 mg/day mg/day L-glutamine in food supplements are considered unlikely to cause adverse health effects.

L-glutamic acid

- In adults (≥18 years), the specified doses of 1000, 2000, 3000, 4000, 5000 and 5500 mg/day L-glutamic acid in food supplements are considered unlikely to cause adverse health effects.

- In adolescents (14 to <18 years), the specified doses of 1000, 2000, 3000, 4000, 5000 and 5500 mg/day L-glutamic acid in food supplements are considered unlikely to cause adverse health effects.

- In children (10 to <14 years), the specified doses 1000, 2000, 3000, and 4000 mg/day L-glutamic acid in food supplements are considered unlikely to cause adverse health effects. The specified doses of 5000 and 5500 mg/day may represent a risk of adverse health effects.

An overview of the conclusions is presented in Tables 6.1 and 6.2.

Table 6.1: An overview of the conclusions for L-glutamine in food supplements.
Green: Estimated exposures to L-glutamine are unlikely to cause adverse health effects.

	L-glutamine						
Doses / Age groups	3500 mg/day	5000 mg/day	8000 mg/day	10000 mg/day	12000 mg/day	15000 mg/day	16500 mg/day
Children (10 to <14 years)							
Adolescents (14 to <18 years)							
Adults (≥18 years)							

Table 6.2: An overview of the conclusions for L-glutamic acid in food supplements.
Green: Estimated exposures to L-glutamic acid are unlikely to cause adverse health effects.
Red: Estimated exposures to L-glutamic acid may represent a risk of adverse health effects.

	L-glutamic acid					
Doses / Age groups	1000 mg/day	2000 mg/day	3000 mg/day	4000 mg/day	5000 mg/day	5500 mg/day
Children (10 to <14 years)						
Adolescents (14 to <18 years)						
Adults (≥18 years)						

7 Data gaps

- **Lack of human toxicity studies on adverse effects as primary outcome of L-glutamine and L-glutamic acid supplementation, with the possibility to establish a dose-response relationship:** The large majority of intervention studies are designed to detect health-protective and health-promoting effects of L-glutamine. Also, most of them are performed in selected patient groups, some of which are not relevant for the use of L-glutamine as a food supplement e.g. emergency unit patients, cancer treatment. There is a need for human studies that are well-designed (randomised, blinded, placebo-controlled, multicenter)
    - with L-glutamine or L-glutamic acid given as a single supplement as the intervention
    - with graded doses
    - of sufficient sample size
    - designed to study long-term effects – i.e. sufficient duration of intervention and sufficient duration of follow-up
    - performed in healthy subjects representative of the general population
- **Lack of data in children and adolescents:** A systematic literature search in children and adolescents with no restriction concerning publication year retrieved no relevant studies, revealing a severe lack of data about potential adverse health effects of L-glutamine and L-glutamic acid in children and adolescents.

- **There is a lack of toxicological studies in rodents** that are
    - performed according to OECD Guidelines or similar
    - with L-glutamine or L-glutamic acid given as a single supplement as the intervention
    - with graded, sufficiently high doses
    - designed to study long-term effects – i.e. sufficient duration of intervention

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Appendix 1

Search strategies for this risk assessment

Search strategy for human studies

Database: Ovid MEDLINE(R) <1946 to May Week 5 2015>, Embase <1974 to 2015 June 03>

1. (glutamine* or glutamic acid* or glutamate*).ti. (88212)
2. glutaminase*.ti. (1882)
3. 1 not 2 (87854)
4. (risk* or safety or adverse or side-effect*1 or hazard* or harm* or neurolog* or psycholog* or behaviour* or behavior* or overweight* weight* or obese* headache* blood pressure* negative or contraindicat* or contra-indicat* or interact* or toxicity or toxic).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, an, tn, dm, mf, dv, kw] (12246030)
5. 3 and 4 (21782)
6. (conference abstract* or letter*).pt. (3605620)
7. 5 not 6 (20562)
8. limit 7 to (danish or english or norwegian or swedish) (19738)
9. limit 8 to human (5260)
10. remove duplicates from 9 (3370)
11. limit 10 to yr="2000 -Current" (2679) (2619 after manual duplicate removal)

Search strategy for animal studies

Database: Embase <1974 to 2015 November 18>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present>

1. glutamine*.ti. (18736)
2. glutamic acid*.ti. (10050)
3. 1 or 2 (28446)
4. glutaminase*.ti. (1961)
5. 3 not 4 (28202)
6. (risk* or safety or adverse or side-effect*1 or hazard* or harm* or neurolog* or psycholog* or behaviour* or behavior* or overweight* weight* or obese* headache* blood pressure* negative or contraindicat* or contra-indicat* or interact* or toxicity or toxic).tw. (10353359)
7. 5 and 6 (4172)
8. (conference abstract* or letter*).pt. (3910848)
9. 7 not 8 (3970)
10. limit 9 to (danish or english or norwegian or swedish) (3769)

11. limit 10 to animals (1393)
12. remove duplicates from 11 (860)

Search strategy for studies in children and adolescents

Database: Embase <1974 to 2016 February 01>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present>

1. (glutamine* or glutamic acid* or glutamate*).ti. (92206)
2. (child* or adolescent* or teenage* or college* or high school).tw. (2946198)
3. 1 and 2 (1147)
4. limit 3 to (danish or english or norwegian or swedish) (995)
5. remove duplicates from 4 (644)