Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

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Risk assessment of "other substances" – L-alanine

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

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Assessed and approved

The opinion has been assessed by the Panel on Nutrition, Dietetic Products, Novel Food and Allergy of the Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM). Kristin Holvik (chair), Livar Frøyland, Margaretha Haugen, Sigrun Henjum, Martinus Løvik, Tonje H. Stea and Tor A. Strand.

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

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Competence of VKM experts

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

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Summary

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

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

For the current report VKM has studied previous risk assessments and performed two systematic literature searches for any published studies assessing negative health effects of L-alanine in humans or animals.

According to information from NFSA, L-alanine is an ingredient in food supplements sold in Norway. NSFA has requested a risk assessment of L-alanine: 3500, 3750, 4000, 4250 and 4500 mg/day. Foods rich in alanine are generally protein rich foods such as meat, dairy products, legumes, fish, nuts, seeds, eggs and whole grains. Based on NHANES III (1988-1994), the overall mean intake of L-alanine from food and food supplements in the United States was 3.6 g/day.

L-alanine is a naturally occurring non-essential α-amino acid belonging to the group of the 20 amino acids that are normal components of food proteins.

L-alanine acts as an intermediary between protein catabolism and carbohydrate synthesis. It can be easily synthesised from the alpha keto acid pyruvate and has close links to several metabolic pathways including glycolysis, gluconeogenesis, and the citric acid cycle. Together with lactate it is capable of generating glucose from muscle protein through gluconeogenesis in the liver. Alanine thus plays a central role in the metabolism of muscle protein and is a key factor in nitrogen metabolism.

Previous reports from the US Institute of Medicine (IOM) 2005, the French Food Safety Agency (AFSSA) 2007 and the Spanish Agency for Food Safety and Nutrition (AESAN) 2012 did not conclude regarding safe doses of L-alanine, but stated that data on adverse effects of L-alanine intake from supplements were not sufficient for a dose-response assessment and establishment of a tolerable upper intake level.

Few studies have assessed health effects of L-alanine supplementation in humans, and these were generally not designed to evaluate potential harmful effects of L-alanine. Most human

experimental studies gave single doses (up to 50 g) or short-term loading doses (e.g. 25 to 45 g/hour during exercise) to study metabolic responses such as ergogenic effects during exercise or prevention of ketosis after fasting. Adverse health effects were not reported except for abdominal discomfort and stomach cramps, nausea and diarrhea after consuming high doses. No studies assessed long-term effects of L-alanine supplementation, and no studies gave doses comparable to the doses under consideration in the present report.

Only one dose-reponse toxicity study in rodents has been found (Chow et al., 1976). In that study, growing Wistar rats were fed up to 20% DL-alanine (a racemic mixture of D- and L-alanine) in their basal diet for 26 weeks, with no effect on liver and kidney weight and no pathological changes in any organs. The study was taken into account due to the otherwise scarce literature on L-alanine toxicity, and could be used since there were no adverse effects at the highest dose tested. The no observed adverse effect level (NOAEL) in mg L-alanine per kg body weight per day was not stated. VKM has therefore estimated the NOAEL using information about average reported feed consumption and average body weights of the animals, and divided by 2 to obtain a NOAEL for L-alanine, arriving at approximately 6450 mg/kg bw/day in male rats and 9700 mg/kg bw/day in female rats. A standard toxicological approach dividing by an uncertainty factor (UF) of 10 for between-species variation and an additional UF of 10 for within-species variation gives the value of 64.5 mg/kg bw per day in females and 97.0 mg/kg bw per day in males, corresponding to approximately 4500 mg/day and 6800 mg/day for a 70 kg man and woman, respectively.

VKM also calculated the margins of exposure (MOE) between the estimated NOAEL and the estimated daily exposures from the five supplement doses given by the NFSA (based on default average body weights for the age groups). MOE were 100 or higher for all five doses in adults. For adolescents 14 to <18 years, the MOE was 88 for the highest dose under consideration. For children 10 to <14 years, MOE ranged from 62 for the highest dose to 80 for the lowest dose.

The VKM considers that these margins are relatively high and are acceptable based on the following considerations: The highest dose tested in growing rats did not cause adverse effects, implying that the "true" NOAEL is unknown and could be considerably higher. Furthermore, L-alanine is a nutrient participating in normal energy metabolism as a substrate for glucose. It is consumed in the magnitude of 3 to 4 g/day on average in the habitual diet, and it has not been associated with harmful effects in humans beyond gastrointestinal effects when consuming very high single doses (50 g, or at a consumption rate of 30 to 45 grams per hour during exercise).

VKM concludes that:

- In adults (≥18 years), the specified doses 3500, 3750, 4000, 4250 and 4500 mg/day L-alanine in food supplements are unlikely to cause adverse health effects.
- In adolescents (14 to <18 years), the specified doses 3500, 3750, 4000, 4250 and 4500 mg/day L-alanine in food supplements are unlikely to cause adverse health effects.

- In children (10 to <14 years), the specified doses 3500, 3750, 4000, 4250 and 4500 mg/day L-alanine in food supplements are unlikely to cause adverse health effects.

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

Short summary

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

- In adults (≥18 years), the specified doses 3500, 3750, 4000, 4250 and 4500 mg/day L-alanine in food supplements are unlikely to cause adverse health effects.
- In adolescents (14 to <18 years), the specified doses 3500, 3750, 4000, 4250 and 4500 mg/day L-alanine in food supplements are unlikely to cause adverse health effects.
- In children (10 to <14 years), the specified doses 3500, 3750, 4000, 4250 and 4500 mg/day L-alanine in food supplements are unlikely to cause adverse health effects.

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

Key words: Alanine, food supplement, adverse health effect, negative health effect, Norwegian Food Safety Authority, Norwegian Scientific Committee for Food Safety, other substances, risk assessment, VKM

Sammendrag på norsk

På oppdrag for Mattilsynet har Vitenskapskomiteen for mattrygghet (VKM) vurdert risiko ved tilsetting av "andre stoffer" i kosttilskudd og energidrikk som selges i Norge. VKM har risikovurdert ulike 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 i kosttilskudd, men også i energidrikker og andre næringsmidler. I disse risikovurderingene har VKM kun vurdert mulige negative helseeffekter, og ikke potensielle gunstige helseeffekter.

I denne rapporten har VKM vurdert helserisiko ved L-alanin i kosttilskudd. Vurderingen er basert på andre tidligere risikovurderinger av aminosyrene, og på vitenskapelige artikler som er funnet i systematiske litteratursøk.

Ifølge informasjon fra Mattilsynet er L-alanin en ingrediens i kosttilskudd som selges i Norge. Oppdraget fra Mattilsynet var å risikovurdere følgende doser av L-alanin i kosttilskudd: 3500, 3750, 4000, 4250 og 4500 mg/dag. Proteinrike matvarer som kjøtt, meieriprodukter, bønner, fisk, nøtter, frø, egg og helkorn har høyt innhold av L-alanin. I en eldre nasjonal kartlegging av befolkningens kosthold i USA (NHANES III, 1988-1994) var gjennomsnittlig inntak av L-alanin fra mat og tilskudd 3,6 g/dag.

L-alanin er en naturlig forekommende ikke-essensiell alfa-aminosyre. L-alanin inngår i en gruppe av 20 aminosyrer som finnes naturlig i proteiner i mat.

L-alanin fungerer som et mellomledd mellom proteinnedbryting og karbohydratsyntese. Den kan lett dannes fra alfaketosyren pyruvat, og er tilknyttet flere metabolske reaksjonsveier som glykolyse, glukoneogenese og sitronsyresyklus. Sammen med laktat nydanner L-alanin glukose fra muskelprotein via glukoneogenese i lever. L-alanin spiller dermed en sentral rolle i omsetningen av muskelprotein og i omsetningen av nitrogen.

Tidligere rapporter fra mattrygghetsorganer i USA (IOM, 2005), Frankrike (AFSSA, 2007) og Spania (AESAN, 2012) konkluderte ikke med noen øvre trygg dose av L-alanin, men uttalte at det ikke var tilstrekkelige data om negative helseeffekter av L-alanin fra kosttilskudd til å etablere en dose-responssammenheng og utlede et tolerabelt øvre inntaksnivå.

Det er få studier som har undersøkt helseeffekter av tilskudd med L-alanin til mennesker, og disse har generelt ikke vært designet for å undersøke skadelige effekter. De fleste eksperimentelle studiene med alanin gitt til mennesker ga høye enkeltdoser på opptil 50 gram, eller «belastningsdoser» på f.eks. mellom 25 og 45 gram per time under fysisk aktivitet. Formålet med studiene har som regel vært å undersøke metabolsk respons, som ergogen effekt under trening eller forebygging av ketose etter faste. Det er ikke beskrevet negative helseffekter utover ubehag i mage-tarm, magekramper, kvalme og diaré etter

inntak av høye doser. Ingen studier har undersøkt langtidseffekter av tilskudd med L-alanin, og ingen studier har gitt doser som tilsvarer de dosene som vurderes i denne rapporten.

Det er funnet én dose-respons toksisitetsstudie i gnagere (Chow et al., 1976). I den studien ble Wistar-rotter i vekst gitt opptil 20 prosent DL-alanin (et racemat bestående av like deler D- og L-isomeren) i basalfôret i 26 uker. Det ble ikke funnet noen effekt på lever- og nyrevekt eller patologiske endringer i noe organ. Siden det er mangelfull litteratur om toksisitet av L-alanin, og siden DL-alanin i høyeste dose ikke medførte negative helseeffekter, ble denne studien benyttet i vurderingen. Forfatterne av studien angir ikke størrelsen på *no observed adverse effect level* (NOAEL), dvs. høyeste daglige dose i mg/kg kroppsvekt som ikke ga negative helseffekter i rottene som fikk 20 prosent DL-alanin. VKM har derfor beregnet NOAEL ved hjelp av opplysninger om gjennomsnittlig inntatt fôrmengde og gjennomsnittlig kroppsvekt hos rottene, og deretter dividert med 2 for å komme frem til en NOAEL for L-alanin på om lag 6450 mg/kg kroppsvekt per dag for hannrotter og 9700 mg/kg kroppsvekt per dag for hunnrotter. En standard toksikologisk tilnærming er å dividere med en usikkerhetsfaktor (UF) på 10 for variasjon mellom arter fulgt av en UF på 10 for interindividuell variasjon, noe som gir verdien 64,5 mg/kg kroppsvekt per dag i hannkjønn og 97.0 mg/kg kroppsvekt per dag i hunnkjønn, tilsvarende om lag 4500 mg/dag og 6800 mg/dag for hhv. en mann og kvinne på 70 kg.

VKM har også beregnet "margin of exposure" (MOE) mellom NOAEL og daglig eksponering fra de spesifikke kosttilskuddosene fra Mattilsynet, ved å benytte verdien 6450 mg L-alanin/kg kroppsvekt per dag som NOAEL, samt standard gjennomsnittskroppsvekt i ulike alderskategorier. For voksne var MOE for alle doser 100 eller høyere. For ungdom 14 til <18 år var MOE 88 for den høyeste kosttilskuddsdosen som er vurdert (4500 mg). For barn 10 til <14 år var MOE mellom 62 for den laveste dosen og 80 for den høyeste dosen.

VKM vurderer at disse marginene er relativt høye og akseptable i lys av følgende betraktninger: Den høyeste dosen L-alanin som ble utprøvd på rotter forårsaket ikke negative helseeffekter, noe som innebærer at den «sanne» NOAEL er ukjent og gjerne kan være betydelig høyere. L-alanin er et næringsstoff som inngår i normal energiomsetning i kroppen som substrat for glukose, den inntas i størrelsesorden 3 til 4 gram per dag i gjennomsnitt i et vanlig kosthold, og den har ikke vært satt i sammenheng med skadelige effekter hos mennesker utover mage-tarmsymptomer ved inntak av svært høye enkeltdoser (50 gram, eller ved en alaninbelastning på 30 til 45 gram per time under en treningsøkt).

Vitenskapskomiteen for mattrygghet (VKM) konkluderer med at:

- For voksne (≥18 år) er det usannsynlig at de spesifiserte dosene på 3500, 3750, 4000, 4250 og 4500 mg/dag L-alanin i kosttilskudd vil forårsake negative helseeffekter.
- For ungdom (14 til <18 år) er det usannsynlig at de spesifiserte dosene på 3500, 3750, 4000, 4250 og 4500 mg/dag L-alanin i kosttilskudd vil forårsake negative helseeffekter.

- For barn (10 til <14 år) er det usannsynlig at de spesifiserte dosene på 3500, 3750, 4000, 4250 og 4500 mg/dag L-alanin i kosttilskudd vil forårsake 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 spesifikke doser av L-alanin i kosttilskudd. VKM konkluderer med at:

- For voksne (≥18 år) er det usannsynlig at de spesifiserte dosene på 3500, 3750, 4000, 4250 og 4500 mg/dag L-alanin i kosttilskudd vil forårsake negative helseeffekter.
- For ungdom (14 til <18 år) er det usannsynlig at de spesifiserte dosene på 3500, 3750, 4000, 4250 og 4500 mg/dag L-alanin i kosttilskudd vil forårsake negative helseeffekter.
- For barn (10 til <14 år) er det usannsynlig at de spesifiserte dosene på 3500, 3750, 4000, 4250 og 4500 mg/dag L-alanin i kosttilskudd vil forårsake negative helseeffekter.

Barn under 10 år inngår ikke i dette oppdraget.

Abbreviations and glossary

Abbreviations

AESAN - Spanish Agency for Food Safety and Nutrition
AFSSA - French Food Safety Agency
bw - body weight
EFSA - European Food Safety Authority
IOM - Institute of Medicine, USA
LOAEL - lowest observed adverse effect level
MOE - margin of exposure
NFSA - Norwegian Food Safety Authority [*Norw.*: Mattilsynet]
NOAEL - no observed adverse effect level
ORS - oral rehydration solution
PBC - primary biliary cirrhosis
RCT - randomised controlled trial
UF - uncertainty factor
UL - tolerable upper intake level
VKM - Norwegian Scientific Committee for Food Safety [*Norw.*: Vitenskapskomiteen for Mattrygghet]
VO_2 max - Maxiumum oxygen consumption
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. The World Helath Organization (WHO) has established the following definition of "adverse effect": a change in morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences (WHO, 1994).

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

Background as provided by the Norwegian Food Safety Authority

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

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

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

The Norwegian Food Safety Authority (NFSA) is working on the establishment of a regulation on the addition of "other substances" to foods at a national level. The regulation will include a list of substances with permitted maximal doses, based on the substances and doses found in products on the Norwegian market. In preparation for a regulation, NFSA has therefore requested the Norwegian Scientific Committee for Food Safety (VKM) to assess the safety of "other substances" found on the Norwegian market. NFSA, in consultation with the industry, has compiled a list of "other substances" found in products marketed in Norway. Only substances with a purity of minimum 50% or concentrated 40 times or more have been included in the list. Substances regulated by other legislations like those for novel foods, food additives, 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-alanine in food supplements at the following doses: 3500, 3750, 4000, 4250 and 4500 mg.

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

The safety assessments for "other substances" present in food supplements shall be carried out for the general population, age 10 years and older.

1 Introduction

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

This risk assessment regards the substances L-alanine per se, and no specific products.

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

According to information from the Norwegian Food Safety Authority (NFSA), L-alanine is an ingredient in food supplements sold in Norway. NFSA has requested a risk assessment of the intake of 3500, 3750, 4000, 4250 and 4500 mg L-alanine per day from food supplements. The total L-alanine exposure from other sources than food supplements is not included in the risk assessment.

Foods rich in L-alanine are generally all protein rich foods and include meat, dairy products, legumes, fish, nuts, seeds, eggs and whole grains. According to NHANES III (1988-1994), the overall mean intake of L-alanine from food and food supplements in the United States was 3.6 g/day (IOM, 2005).

Alanine is a non-essential α-amino acid. Alanine occurs in a D- and L-form where L-alanine is the common form and the D-form occurs in some bacteria and peptide antibiotics.

Alanine participates in energy metabolism as an intermediary between protein catabolism and carbohydrate synthesis (Felig, 1973). Alanine can be easily formed and has close links to a number of metabolic pathways including glycolysis, gluconeogenesis, and the citric acid cycle. The glucose synthesised from alanine by gluconeogenesis in the liver is used for muscular contraction, erythrocyte metabolism and brain function. Together with glutamate and aspartate, alanine is a key amino acid in systemic nitrogen homeostasis, and is one of the principal amino acids released from muscle (Elia, 1991). The release and clearance of alanine is high, even in cirrhotic patients (Schricker et al., 1995).

2 Hazard identification and characterisation

2.1 Literature

This risk assessment is based on previous risk assessments of L-alanine, as well as scientific papers retrieved from systematic searches in literature published before 14 September 2016 (human studies) and 28 October 2016 (animal studies) aimed at retrieving publications on adverse effects caused by L-alanine.

2.1.1 Previous risk assessments

The safety of L-alanine has been discussed in previous reports from the US Institute of Medicine (IOM) 2005, The French food Safety Agency (AFSSA) 2007 and the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) 2012.

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

2.1.1.2 Protein intake: dietary intake, quality, requirements and recommendations. French Food Safety Agency (AFSSA). France, 2007

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

2.1.2 Literature search

For the current report, systematic literature searches were performed in MEDLINE and EMBASE with no restriction on year of publication, in order to retrieve publications on adverse effects caused by L-alanine. Both databases were searched to ensure comprehensive study retrieval. The literature search for human studies was conducted on 14 September 2016, and the search for animal studies was conducted on 28 October 2016. The search strategies are outlined in Appendix 1.

2.1.2.1 Publication selection and data extraction

2.2 General information

2.2.1 Chemistry

L-alanine is a non-essential amino acid that is synthesised from pyruvate and the branched chain amino acids valine, leucine, and isoleucine. The molecular formula is $C_3H_7NO_2$, and the CAS number is 56-41-7. The structural formula is shown in figure 2.2.1-1.

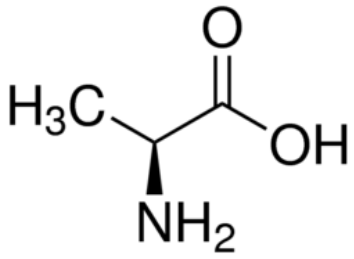

Figure 2.2.1-1: Structural formula of L-alanine.

2.2.2 Occurrence

L-alanine is a non-essential amino acid that can be synthesised by all known animals and plants. Hence, alanine is found in a wide variety of foods, but is particularly concentrated in meats as it is produced in large quantities by skeletal muscle. Animal sources of alanine include meat, seafood, caseinate, dairy products, eggs, fish, gelatin, and lactalbumin from milk. Plant sources include beans, nuts, seeds, soy, whey, brewer's yeast, brown rice, bran, corn, whole grains and legumes.

2.3 Absorption, distribution, metabolism and excretion

Absorption, distribution, metabolism and excretion of alanine is shared between human and relevant animal models. Most food proteins contains between 4 and 8% alanine (Friedman, 1999). Alanine is ingested as part of larger proteins and to some extent as a free amino acid. Monomers of alanine or alanine contained in di- or tri-peptides are released from larger proteins by hydrolysis in the gastrointestinal tract by action of enzymes such as pepsin, carboxy peptidase, pancreatic endopeptidase and alanine amino peptidase. Free alanine and di- and tri peptides are taken up in the intestine epithelial cells through the B° co-transporter (ASCT2), and SLC15A1/and PepT1, respectively. Monomeric alanine is released form the di- and tri peptides in the epithelium cells and transported into the portal vein by a sodium-amino transporter system called ATA2/ASCT1 and further transported to the liver. Upon starvation uptake of alanine is enhanced by increased transcription and expression of the transporters (Muniz et al., 1993). Aside from its role in protein synthesis, alanine is second amino acid only to glutamine in abundance in circulation. Normal concentrations are typically between 270 and 500 µM and increase significantly after a meal (Tsai and Huang, 1999). Qualitative uptake of alanine in peripheral tissues and cells is facilitated by tissue-specifically

expressed sodium-dependent transporters and quantitative uptake depends on the endogenous concentration of alanine in individual cells (Sugawara et al., 2000; Varoqui et al., 2000).

In addition to being a constituent part of all proteins in the human body, alanine is a precursor by transamination for a number of amino acids including glutamate, glutamine, glycine, phenylalanine and serine. As a result pyruvate is formed and can be used to feed the citric acid cycle and in this way fuel oxidative phosphorylation and ATP synthesis. Moreover, alanine acts a precursor for heme and glyoxalate. In the latter the amino group of alanine is transferred to glyoxalate where glyoxalate is a crucial precursor in the synthesis of glycine. When glycine is not produced glyoxalate may simply be converted to the citric acid cycle intermediate oxalo acetate.

Alanine can be produced from the branched chain amino acids leucine, isoleucine and valine and the alpha-keto acid pyruvate. Pyruvate and alanine play key roles in the so called glucose–alanine cycle between peripheral tissues and liver. In muscle and other tissues that degrade amino acids for fuel, amino groups are collected in the form of glutamate by transamination. Glutamate can then transfer its amino group through the action of alanine aminotransferase to pyruvate, a product of muscle glycolysis, forming alanine and α-ketoglutarate. The alanine formed is passed into the blood and transported to the liver. A reverse of the alanine aminotransferase reaction takes place in liver. Pyruvate regenerated forms glucose through gluconeogenesis, which returns to muscle or other tissues requiring glucose through the circulation system. Glutamate formed in the liver enters mitochondria and degrades into ammonia through the action of glutamate dehydrogenase, which in turn participates in the urea cycle to form urea.

Because transamination reactions are readily reversible and pyruvate pervasive it explains why alanine can be easily formed and has close links to a number of metabolic pathways including glycolysis, gluconeogenesis, and the citric acid cycle. This means that alanine together with glutamate and aspartate is crucial in systemic nitrogen homeostasis as it is a substrate of the main aminotransferases that directly connect the amino acids to the intermediary keto acids pyruvate, alpha keto glutarate and oxaloacetate. Moreover, the glucose obtained from alanine by gluconeogenesis in the liver is used for muscular contraction, erythrocyte metabolism and brain function further stressing the crucial role of this amino acid.

2.4 Toxicological data/Adverse effects

2.4.1 Human studies

2.4.1.1 Studies discussed in previous reports

2.4.1.2 Publications retrieved in the systematic literature review

2.4.1.3 Interactions

There was no information concerning interactions in the literature reviewed in the present risk assessment. The absence of information in the literature does not document an absence of interactions.

2.4.1.4 Allergic sensitisation (including adjuvant effects)

2.4.2 Animal studies

Two older animal studies have been cited in previous reports:

- In a study on the toxicity and antagonism of amino acids for weanling rats published by Sauberlich in 1961 (Sauberlich HE, J Nutr 1961; 75: 61-72), alanine exerted no adverse effect on rats fed 5% in a low-protein diet.
- In a study published by Adkins, Sudne and Harper in 1962 (Adkins et al., Poultry Science 1962; 41: 1382-8), L-alanine levels up to 4% did not depress growth in growing chicks fed an adequate diet.

In the systematic literature search for the current report, one publication reporting a 26-week feeding study with alanine to weanling rats was obtained (Chow et al., 1976). To our knowledge, this is the only dose-response toxicity study that has been performed with graded oral doses of alanine. Note that DL-alanine was the substance given in this study. The study is described in detail below.

Alanine: A toxicity study. Chow et al., *Toxicol Appl Pharmacol* 1976

In this 6-month (26 weeks) feeding study, male and female Wistar rats of weanling age were acclimated for 1 week before being randomly divided into four groups with eight male and eight female rats in each group (Chow et al., 1976). They were fed a basal ration containing 0, 5, 10, or 20% DL-alanine. DL-alanine denotes a racemic mixture (i.e. a 1:1 mixture of the D- and L-isomer) so we here assume that L-alanine constituted half the dose given. The study is taken into account in the current risk assessment due to the otherwise scarce literature on L-alanine toxicity, and is applicable since there were no adverse effects at the highest dose tested.

Concerning blood chemistry changes, alanine caused a decrease in plasma pyruvate and triglycerides. Ammonia increased in males only, while lactate decreased in females only. Concerning growth depression, the rats fed the highest alanine dose gained less weight compared with the rats fed lower alanine doses. Gross examination for pathological changes of all organs at necropsy after 26 weeks showed no abnormalities and lesions, and there was no effect on liver or kidney weight. The authors concluded that alanine rations of up to 20% of the feed did not appear harmful to health, but added that periods of nutritional stress such as pregnancy or lactation had not been studied.

The magnitude of the NOAEL was not stated, but the paper provided the overall mean daily food intake in the study groups, determined every 2nd week. The overall final weights were stated to be 300 g in females and 600 g in males, of which 86% and 80% was gained halfway through the study, respectively. Based on this information, we have estimated that the dose of DL-alanine consumed in the 20% alanine diet was in the magnitude of 12900 mg/kg bw/day in the male rats and 19400 mg/kg bw/day in female rats. L-alanine, constituting half the dose, was thus about 6450 mg/kg bw/day in female rats and 9700 mg/kg bw/day in male rats consuming the 20% alanine diet.

2.4.3 Vulnerable groups

VKM has not retrieved any information about particular vulnerable groups to excess doses of L-alanine. As described above (Ribeiro Junior Hda and Lifshitz, 1991), a supplementation with added alanine to a standard WHO oral rehydration solution to infant boys produced no negative effects. There have been no reported studies in pregnant or lactating women.

2.5 Summary of hazard identification and characterisation

Previous reports (IOM, 2005; AFSSA, 2007; AESAN, 2012) did not conclude regarding safe doses of L-alanine, but stated that data on adverse effects of L-alanine intake from supplements were not sufficient for a dose-response assessment and establishment of a tolerable upper intake level.

Few studies have assessed health effects of L-alanine supplementation in humans, and these were generally not designed to evaluate potential harmful effects of L-alanine. Most human studies gave high single doses (up to 50 g) or short-term loading doses (e.g. 25 to 45 g/hour during exercise) to study metabolic responses such as ergogenic effects during exercise or prevention of ketosis after fasting. Adverse health effects have not been reported except for abdominal discomfort and stomach cramps, nausea and diarrhea after consuming high doses. Stomach cramps could be attributed to the high intake of a single amino acid while the nausea could be attributed to increased production of beta-hydroxybutyrate, an intermediate of keto acid synthesis. No studies assessed long-term effects of L-alanine supplementation, and no studies gave doses comparable to the doses under consideration in the present report.

Only one dose-reponse toxicity study in animals has been found (Chow et al., 1976). In that study, growing Wistar rats were fed up to 20% DL-alanine in their basal diet for 26 weeks, with no effect on liver and kidney weight and no pathological changes in any organs. The authors concluded that alanine rations of up to 20% of the feed did not appear harmful to health, but this study did not cover periods of nutritional stress such as pregnancy or lactation.

The paper provided the overall mean daily food intake in the study groups, determined every second week. The overall final weights were reported to be 300 g in females and 600 g in

males, of which 86% and 80% was gained halfway through the study, respectively. Using halfway average body weight we have estimated that the dose of DL-alanine consumed in the 20% alanine diet was in the magnitude of 12900 mg/kg bw/day in the male rats and 19400 mg/kg bw/day in the female rats. L-alanine, constituting half the dose, was thus about 6450 mg/kg bw/day in male rats and 9700 mg/kg bw/day in female rats consuming the 20% alanine diet.

For comparison with the L-alanine doses under consideration in the current report, dividing by an uncertainty factor (UF) of 10 for between-species variation and an additional UF of 10 for within-species variation, in line with standard toxicological risk assessment, gives the value of 64.5 mg/kg bw/day in males and 97.0 mg/kg bw/day in females. This corresponds to 4500 mg/day and 6800 mg/day for a 70 kg man and woman, respectively. Note that in the case of L-alanine this estimate is conservative since it is derived using UF 100 while:

a) the highest dose tested in the study in growing rats by Chow et al. (1976) did not cause adverse health effects, implying that the "true" NOAEL is unknown and could be considerably higher

b) L-alanine is a nutrient participating in normal energy metabolism as a substrate for glucose, and it is consumed in the magnitude of 3 to 4 g/day on average in the habitual diet

c) L-alanine has not been shown to be associated with harmful effects in humans in the literature, except for nausea and abdominal discomfort when consuming very high single doses (50 g, or at a consumption rate of 30 to 45 grams per hour during exercise)

On the other hand, the uncertainty is large because of the scarce literature, and human experimental studies have almost exclusively been performed with single or short-term loading doses. The longest duration of supplementation was an 8-week uncontrolled supplementation study with L-alanine to three patients with severe liver disorder, which is of little relevance for the general population. No studies on chronic alanine consumption in humans or animals have been identified.

3 Exposure / Intake

Exposures of L-alanine were estimated from the intake of food supplements. For food supplements, the intake was estimated for the age groups 10 to <14 years, 14 to <18 years and adults (≥18 years).

3.1 Food supplements

The Norwegian Food Safety Authority requested VKM to perform a risk assessment of 3500, 3750, 4000, 4250 and 4500 mg/day of L-alanine in food supplement for children (10-17 years) and adults. The default body weights for age groups determined by EFSA were used: 10 to <14 years = 43.4 kg, 14 to <18 years = 61.3 kg and adults = 70.0 kg. The exposures per kg bw are given in Table 3.1-1.

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

Groups	L-alanine, daily doses (mg)	Body weight (kg)	Exposure (mg/kg bw per day)
Children (10 to <14 years)	3500, 3750, 4000, 4250 and 4500	43.4	80.7, 86.4, 92.2, 97.9 and 103.7
Adolescents (14 to <18 years)	3500, 3750, 4000, 4250 and 4500	61.3	57.1, 61.2, 65.3, 69.3 and 73.4
Adults (≥18 years)	3500, 3750, 4000, 4250 and 4500	70.0	50.0, 53.6, 57.1, 60.7 and 64.3

3.2 Other sources

Based on the NHANES III (1988-1994), the overall mean intake of L-alanine from food and food supplements in the United States was 3.6 g/day. The 99th percentile of intake was highest in adult men; 8.5 g/day in men aged 51-70 years and 8.3 g/day in men aged 19-30 years (IOM, 2005).

4 Risk characterisation

The doses received from NFSA for assessment were 3500, 3750, 4000, 4250 and 4500 mg/day L-alanine in food supplements, and the estimated exposures for adults, adolescents and children 10 years and older derived from these dose levels are given in chapter 3.

We found no data indicating that children and adolescent are more vulnerable than adults for L-alanine. No tolerance level is set for L-alanine specifically for children or adolescents.

We have calculated the margins of exposure (MOE), defined as the NOAEL divided by the magnitude of exposure for each dose and age category (based on Table 3.1-1), using the estimated NOAEL for L-alanine (the NOAEL for DL-alanine divided by 2) for males that we have estimated from the study by Chow et al. (1976), i.e. 6450 mg/kg bw per day.

The margins of exposure are shown in Table 4-1.

Table 4-1 Calculated margins between the NOAEL from a 26-week toxicity study in rats and the exposure to L-alanine from food supplements for the age groups covered by this risk assessment

Groups	3500 mg/day	3750 mg/day	4000 mg/day	4250 mg/day	4500 mg/day
Children (10 to <14 years) (43.4 kg)	80	75	70	66	62
Adolescents (14 to <18 years) (61.3 kg)	113	105	99	93	88
Adults (≥18 years) (70.0 kg)	129	120	113	106	100

For adults, MOE for all doses exceed 100. In adolescents (14 to <18 years; default body weight 61.3 kg), MOE are close to 100, while it is 88 for the highest dose under consideration. For children (10 to <14 years; default body weight 43.4 kg), the MOE for the highest dose (4500 mg/day) represents a factor of 62.

In this risk characterisation VKM considers that:

- The margins of exposure using the results from the toxicity study by Chow et al. (1976) are relatively high; the lowest MOE (for the highest dose in children) corresponds to UF 10 for between-species extrapolation multiplied by UF 6 for interindividual variation
- The highest dose tested produced no adverse effects in growing rats, implying that the magnitude of the "true" NOAEL is unknown and could be considerably higher
- L-alanine is a nutrient participating in normal energy metabolism as a substrate for glucose, and it is consumed in the magnitude of 3 to 4 g/day on average in the habitual diet

- Small and short-term experimental studies giving very high single doses or loading doses to humans (50-100 g as single doses or 30 to 45 g per hour during exercise) have not reported any negative health effects beyond gastrointestinal symptoms (abdominal pain and discomfort, nausea, diarrhea)

Based on this, VKM considers that L-alanine at the specified doses 3500, 3750, 4000, 4250 and 4500 mg/day are unlikely to cause adverse health effects in all age groups covered by the current risk assessment.

5 Uncertainties

Only one animal study, performed in rats, was found that met the inclusion criteria in this risk assessment. The substance given was DL-alanine. Since the publication did not provide the magnitude of the NOAEL, we have attempted to estimate this based on imprecise measures of average amounts of food consumed and average body weights of the animals. There are uncertainties and approximations in every step of the considerations made.

The daily exposure per kg body weight of the L-alanine supplement doses given by the NFSA has been estimated using default average body weights for three age groups (adults, adolescents, and children), while the true exposure and the corresponding potential risk will be higher for indivduals with lower body weight.

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-alanine in food supplements at the doses 3500, 3750, 4000, 4250 and 4500 mg/day for the general population, ages 10 years and above.

No particular vulnerable groups for L-alanine supplements have been identified. No data have been found indicating that children or adolescents are more vulnerable than adults for L-alanine and no tolerance level is set for L-alanine specifically for children or adolescents. The conclusions are therefore based on the assumption of similar tolerance for children and adolescents as for adults.

VKM concludes that:

- In adults (≥18 years), the specified doses 3500, 3750, 4000, 4250 and 4500 mg/day L-alanine in food supplements are unlikely to cause adverse health effects.
- In adolescents (14 to <18 years), the specified doses 3500, 3750, 4000, 4250 and 4500 mg/day L-alanine in food supplements are unlikely to cause adverse health effects.
- In children (10 to <14 years), the specified doses 3500, 3750, 4000, 4250 and 4500 mg/day L-alanine in food supplements are unlikely to cause adverse health effects.

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

Table 6-1: An overview of the conclusions for L-alanine in food supplements.
Green: Estimated exposures to L-alanine are unlikely to cause adverse health effects.

	L-alanine				
Doses Age groups	3500 mg/day	3750 mg/day	4000 mg/day	4250 mg/day	4500 mg/day
Children (10 to <14 years)					
Adolescents (14 to <18 years)					
Adults (≥18 years)					

7 Data gaps

- There is a lack of studies designed to detect adverse health effects of L-alanine supplementation in humans
- The few human studies gave acute single doses or loading doses. Human studies on adverse effects after subchronic or chronic oral exposure to L-alanine are lacking.
- There is a lack of dose-response toxicity studies in animals.
- There are especially few studies on negative health effects related to L-alanine in children and adolescents, and no studies are found that include effects of L-alanine in lactating or pregnant women.
- No adequate studies on chronic toxicity and carcinogenicity are available. Only one subchronic dose-response toxicity study in rats was found. This study gave DL-alanine, and the NOAEL was not reported.

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Appendix 1

Search strategies for this risk assessment

Search strategy human studies

Database: Embase <1974 to 2016 September 13>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present>

1. alanine*.ti. (16574)
2. alanine aminotransferase.ti. (2565)
3. 1 not 2 (14009)
4. (risk* or safety or adverse or side-effect*1 or hazard* or harm* or negative or contraindicat* or contra-indicat* or interact* or toxicity or toxic).tw. (10259252)
5. 3 and 4 (2308)
6. (conference abstract* or letter* or editorial*).pt. (5171367)
7. 5 not 6 (2239)
8. limit 7 to (danish or english or norwegian or swedish) (2188)
9. limit 8 to human (580)
10. remove duplicates from 9 (367)

Search strategy animal studies

Database: Embase <1974 to 2016 October 27>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present>

1. alanine*.ti. (16391)
2. alanine aminotransferase.ti. (2595)
3. 1 not 2 (13796)
4. (risk* or safety or adverse or side-effect*1 or hazard* or harm* or negative or contraindicat* or contra-indicat* or interact* or toxicity or toxic).tw. (10393048)
5. 3 and 4 (2326)
6. (conference abstract* or letter* or editorial*).pt. (5207647)
7. 5 not 6 (2256)
8. limit 7 to (danish or english or norwegian or swedish) (2205)
9. limit 8 to animals (555)
10. remove duplicates from 9 (395)