Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

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Risk assessment of "other substances" – L-threonine

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

Report from the Norwegian Scientific Committee for Food Safety (VKM) 2017:10
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Risk assessment of "other substances" - L-threonine

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Assessed and approved

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

The present report is a risk assessment of specified doses of L-threonine in food supplements, and it is based on previous risk assessments and articles retrieved from literature searches.

According to information from NFSA, L-threonine is an ingredient in food supplements sold in Norway. NSFA has requested a risk assessment of 1000, 1200, 1500, 2000 and 2400 mg/day of L-threonine from food supplements.

L-threonine is an essential amino acid not known to cause any adverse health effects. Previous reports do not indicate a tolerable upper intake level, apart from an approval of a dose of 1150 mg/day by the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN). Long-term studies in humans were not found. The only available human studies were: a small uncontrolled one-year pilot study with doses ranging from 0.5 to 2.5 g/day, one eight-week randomised controlled trial (RCT) using a dose of 7.5 g/day, and two 2-week RCTs using doses of 6 and 4.5 g/day. No adverse effects (diary method of registration of adverse effects) were reported in the eight-week clinical trial, and the only adverse effects observed in the two-week trials were one case of indigestion and one case of diarrhoea. A four-week rodent toxicity study indicated a no observed adverse effect level (NOAEL) of 854.3 mg/kg bw per day (only dose tested, no adverse effects observed).

The value used for comparison with the estimated exposure in the risk characterisation is the NOAEL defined in an 8-week randomised placebo controlled study in humans, 7500 mg/day. For a 70-kg individual, this corresponds to 107 mg/kg bw per day. Two human two-week studies and a small one-year pilot study support the notion that this dose will be well tolerated. The overall mean threonine intake according to NHANES III (3 g/day) is slightly larger than the doses requested for evaluation in the present risk assessment.

No studies in children (10 to <14 years) and adolescents (14 to <18 years) were identified. Based on the included literature there was no evidence indicating that age affects the

tolerance for relevant doses of threonine. Therefore, in this risk characterisation a tolerance as for adults, based on body weight, was assumed for these age groups.

VKM concludes that:

- In adults (≥18 years), the specified doses 1000, 1200, 1500, 2000 and 2400 mg/day L-threonine in food supplements are unlikely to cause adverse health effects.
- In adolescents (14 to <18 years), the specified doses 1000, 1200, 1500, 2000 and 2400 mg/day L-threonine in food supplements are unlikely to cause adverse health effects.
- In children (10 to <14 years), the specified doses 1000, 1200, 1500, 2000 and 2400 mg/day L-threonine 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-threonine in food supplements. VKM concludes that:

- In adults (≥18 years), the specified doses 1000, 1200, 1500, 2000 and 2400 mg/day L-threonine in food supplements are unlikely to cause adverse health effects.
- In adolescents (14 to <18 years), the specified doses 1000, 1200, 1500, 2000 and 2400 mg/day L-threonine in food supplements are unlikely to cause adverse health effects.
- In children (10 to <14 years), the specified doses 1000, 1200, 1500, 2000 and 2400 mg/day L-threonine in food supplements are unlikely to cause adverse health effects.

Key words: Threonine, 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 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.

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

Ifølge informasjon fra Mattilsynet er L-treonin en ingrediens i kosttilskudd som selges i Norge. Oppdraget fra Mattilsynet var å risikovurdere følgende doser av L-treonin i kosttilskudd: 1000, 1200, 1500, 2000 og 2400 mg/dag.

L- treonin er en essensiell aminosyre uten kjente negative helseeffekter. Tidligere rapporter støtter oppfatningen av at treonin har lav toksisitet, men angir ikke et øvre tolerabelt inntaksnivå bortsett fra at de spanske vitenskapskomiteen for mattrygghet og ernæring (AESAN) har godkjent en dose på 1150 mg/dag. Det er kun funnet fire humanstudier; en liten ukontrollert pilotstudie med ett års varighet og doser fra 0,5 til 2,5 g/dag, en 8-ukers randomisert, placebokontrollert studie med en dose på 7,5 g/dag, og to randomiserte placebokontrollerte studier av 2 ukers varighet med doser på 6 og 4,5 g/dag. Det ble ikke rapportert om negative helseeffekter (dagbok-metode for registrering) i den kliniske 8-ukers studien, og de eneste rapporterte negative helseeffekter i 2-ukersstudiene var ett tilfelle av fordøyelsesproblemer og ett tilfelle med diaré. I en 4 uker lang toksisitetsstudie i gnagere ble det indikert en NOAEL (no observed adverse effect level) på 854,3 mg/kg kroppsvekt per dag (eneste dose testet).

Som basis for vår sammenligningsverdi ("value of comparison») valgte vi å benytte 7500 mg/dag fra den åtte uker lange randomiserte, placebokontrollerte studien i mennesker. Dette svarer til 107 mg/kg kroppsvekt per dag hos en person på 70 kg. To 2-ukers humanstudier og en liten pilotstudie i mennesker støtter opp under vurderingen av at en slik dose vil bli godt tolerert.

Gjennomsnittlig inntak av treonin (3 g/dag i følge data fra NHANES III, USA) er litt større enn de dosene VKM er bedt om å vurdere for kosttilskudd.

Det ble ikke avdekket noen studier med barn (10 til <14 år) eller ungdom (14 til <18 år). Det er imidlertid ingen indikasjoner på at alder påvirker toleransen for treonin i de inkluderte

studiene. I denne risikovurderingen er det derfor antatt at barn og ungdom har samme toleranse, per kroppsvekt, som voksne. Vitenskapskomiteen for mattrygghet (VKM) konkluderer med at:

- For voksne (≥18 år) er det usannsynlig at de spesifiserte dosene på 1000, 1200, 1500, 2000 and 2400 mg/dag L-treonin i kosttilskudd vil forårsake negative helseeffekter.
- For ungdom (14 til <18 år) er det usannsynlig at de spesifiserte dosene 1000, 1200, 1500, 2000 and 2400 mg/dag L-treonin i kosttilskudd vil forårsake negative helseeffekter.
- For barn (10 til <14 år) er det usannsynlig at de spesifiserte dosene på 1000, 1200, 1500, 2000 and 2400 mg/dag L-treonin 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-treonin i kosttilskudd. VKM konkluderer med at:

- For voksne (≥18 år) er det usannsynlig at de spesifiserte dosene på 1000, 1200, 1500, 2000 and 2400 mg/dag L-treonin i kosttilskudd vil forårsake negative helseeffekter.
- For ungdom (14 til <18 år) er det usannsynlig at de spesifiserte dosene på 1000, 1200, 1500, 2000 and 2400 mg/dag L-treonin i kosttilskudd vil forårsake negative helseeffekter.
- For barn (10 til <14 år) er det usannsynlig at de spesifiserte dosene på 1000, 1200, 1500, 2000 and 2400 mg/dag L-treonin i kosttilskudd vil forårsake negative helseeffekter.

Abbreviations and glossary

Abbreviations

AESAN - Spanish Agency for Food Safety and Nutrition
ASC - alanine/serine/systeine
ASCT - alanine/serine/cysteine transporter
ATA - amino acid transporter
bw - body weight
CoA - coenzym A
EAT - excitatory amino acid-Na^+ cotransporter
EFSA - European Food Safety Authority
EMBASE - a database that registrerers references from articles in more than 7600 medical journals from different countries, with a particular coverage of Western European publications
IOM - Institute of Medicine, USA
LAT - system L neutral amino acid transporter (LAT; LAT1, LAT2, LAT3, or LAT4)
LOAEL - lowest observed adverse effect level
MEDLINE - the U.S. National Library of Medicine® (NLM) premier bibliographic database that contains more than 23 million references to journal articles in life sciences with a concentration on biomedicine
MOE - margin of exposure
NFSA - Norwegian Food Safety Authority [*Norw.*: Mattilsynet]
NHANES - National Health and Nutrition Examination Survey, USA
NOAEL - no observed adverse effect level
OECD - Organisation for Economic Co-operation and Development
PepT - peptide transporter
RCT - randomised controlled trial
SLC15A - Solute Carrier 15 (SLC15) family of peptide transporters is a group of membrane transporters including SLC15A1 (PEPT1) and SLC15A2 (PEPT2)
SMA15 - Sequential Multiple Analysis 15 - a clinical chemistry test battery
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. 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-threonine in food supplements at the following doses: 1000, 1200, 1500, 2000 and 2400 mg/day.

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 substance L-threonine 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-threonine is an ingredient in food supplements sold in Norway. NFSA has requested a risk assessment of the intake of 1000, 1200, 1500, 2000 and 2400 mg L-threonine per day from food supplements. The total threonine exposure from other sources than food supplements is not included in the risk assessment.

L- threonine is an essential/indispensable, neutral amino acid. Threonine is needed for the synthesis of proteins, and practically all human proteins and peptides contain threonine.

In humans threonine is mainly catabolised by threonine dehydratase to NH_3 and 2-ketobutyrate and further downstream to propionyl-CoA, which is rapidly and irreversibly converted to CO_2 in the Krebs cycle. An alternative, minor pathway (<10%) is initiated by threonine dehydrogenase before cleavage to form glycine and acetyl-CoA. By this latter pathway threonine will supply substrates for both the glucose and lipid synthesis (Darling et al., 2000).

All protein sources contain threonine. Based on the threonine content of soy protein, a protein intake of 100 g/day is estimated to provide about 4 g of L-threonine. According to the third National Health and Nutrition Examination Survey (NHANES III, 1988-1994), the overall mean intake of L-threonine from food and food supplements in the United States was 3 g/day. Recommended daily intake is 15 mg/kg bw per day (WHO, 2007), corresponding to 1050 mg for a 70-kg person. Adequate amounts of threonine will be consumed when total protein intake meets recommendations.

2 Hazard identification and characterisation

2.1 Literature

This risk assessment is based on previous risk assessments of L-threonine, as well as scientific papers retrieved from systematic literature searches. The literature searches aimed at retrieving human as well as animal studies on adverse effects caused by L-threonine.

2.1.1 Previous risk assessments

Risks related to L-threonine have previously been evaluated by the Institute of Medicine (IOM) in USA in 2005, EFSA in 2006 and 2008, VKM in 2011 and the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) for use in food supplements in 2012 (AESAN, 2012; IOM, 2005; VKM, 2011).

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

Risk assessment body, country, publication year	Objective	Conclusion	Suggested doses
IOM, USA 2005	To establish dietary reference intakes for L-threonine, and other nutrients. Includes a discussion of potential toxicity.	L-threonine: the data on possible adverse effects of L-threonine intake from supplements were not available for a dose–response assessment and derivation of a UL in apparently healthy humans	L-threonine: Not established
EFSA, 2006	To evaluate the safety of a group of flavouring agents, including L-threonine.	The available data on threonine do not give rise to safety concern with respect to genotoxicity.	L-threonine: Not established
EFSA, 2008	To evaluate the safety of a group of flavouring agents, including L-threonine.	Threonine [FL-no: 17.021] would not give rise to safety concerns at the estimated levels of intake arising from its use as a flavouring substance. Threonine is expected to be metabolised to innocuous products.	L-threonine: Not established
VKM, Norway, 2011	To qualitatively rank 30 amino acids according to high, medium or low risk	Threonine was grouped as "moderate risk"	Threonine: Not established

Risk assessment body, country, publication year	Objective	Conclusion	Suggested doses
AESAN, Spain, 2012	The use of threonine as a food supplement was assessed	The AESAN report largely builds on the IOM 2005 report, and finds the proposed dose acceptable from a safety viewpoint without citing specific considerations. Additionally, the proposed dose is in line with the daily requirement of L-threonine established by WHO.	L-threonine 1150 mg/day acceptable

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

A study in which rats fed 5 percent threonine added to a 10 percent casein diet, showed reduced weight gain, but no changes in liver weight, hepatic DNA, RNA, or protein content compared to controls fed casein alone (Muramatsu et al., 1971). In weanling pigs, adding 0.5, 1, 2, or 4% L-threonine to a 20% crude protein diet did not change weight gain, food intake, and gain: feed ratios in comparison to the controls (Edmonds and Baker, 1987; Edmonds et al., 1987). Evidence indicates that excess threonine is converted to carbohydrate, liver lipids, and carbon dioxide (Yamashita and Ashida, 1971).

No data were found by IOM on apparently healthy humans given oral L-threonine supplements. L-threonine has also been used clinically with the aim of increasing glycine concentrations in the cerebro-spinal fluid of patients to reduce spasticity. When given in amounts of 4.5 to 6.0 g/day for 14 days, no adverse clinical effects were noted in such patients (Growdon et al., 1991).

IOM (2005) found that the data on possible adverse effects of L-threonine intake from supplements were not available for a dose–response assessment and derivation of a tolerable upper intake level (UL) in apparently healthy humans.

EFSA, 2006 and 2008

The available data on threonine do not give rise to safety concerns with respect to genotoxicity. Threonine is expected to be metabolised to innocuous products (EFSA, 2006; EFSA, 2008).

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). Amino acids were to be grouped into one of three categories, high risk, moderate

risk, and low risk. It was emphasised that the VKM report from 2011 has several limitations and can only be regarded as an initial screening and not as a risk assessment of the many amino acids.

In the absence of human studies on negative health effects, threonine was placed in the group 'moderate risk'. The justification was that there is little documentation regarding health effects of threonine, and that amino acids in general are bioactive components.

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

The AESAN report largely cites the IOM (2005) evaluation (AESAN, 2012). A proposal of a maximum dose of 1150 mg/day for use as a food supplement was found acceptable from the safety viewpoint, also because it is in line with the daily requirement of L-threonine established by WHO (2007).

2.1.2 Literature search

Literature searches were performed in MEDLINE and EMBASE in order to retrieve publications on adverse effects caused by L-threonine. Both databases were searched to ensure comprehensive study retrieval. The literature search was conducted 5 April 2016, and included human studies in general. Since studies in children and adolescents were lacking, an additional literature search for these groups was performed 13 October. Further, a literature search for relevant animal studies was performed 16 September 2016.

The strategies for the searches are outlined in Appendix 1.

2.1.2.1 Publication selection and data extraction

The literature search for human studies identified 427 articles, and the search for studies in children and adolescents 17 studies. The search for animal studies identified 440 titles. In the primary screening, titles and abstracts of all unique publications retrieved were independently screened against the inclusion criteria.

Inclusion criteria:

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

In vitro studies were not included, but relevant titles were read in order to find background information regarding potential toxic properties of L-threonine.

Data from studies on acetyl-threonine were used only as supportive information.

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

The papers that passed the primary screening were reviewed in full against the same inclusion criteria by the author author of this report.

For the general human studies search, the first screening resulted in 3 full text articles, all included after full text reading, with the addition of one paper found by hand search. From the search for studies in children and adolescents, no studies were included after the first screening. One animal study was included after the first screening and full text reading (see Figure 2.1.2.1-1).

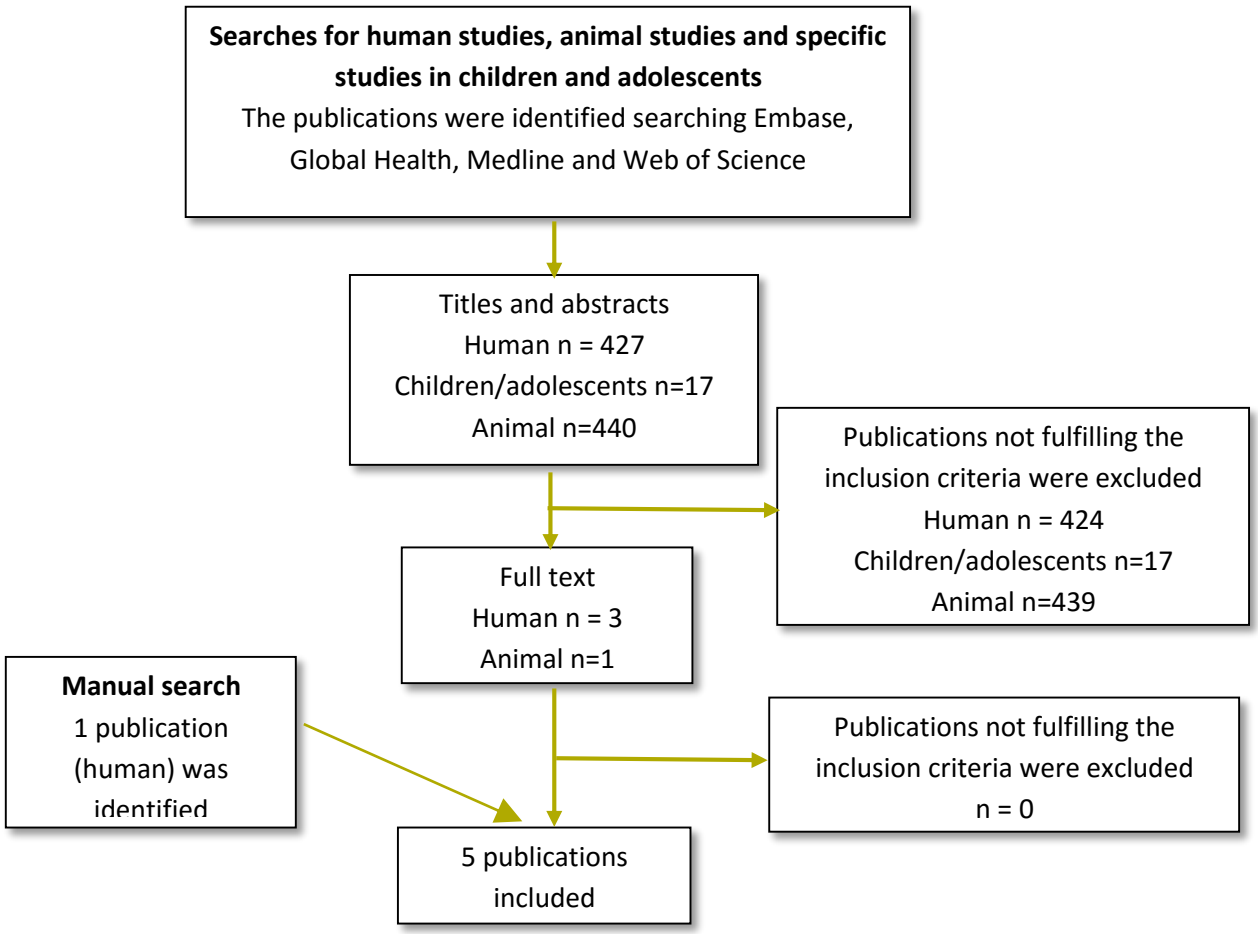

Figure 2.1.2.1-1: Flowchart for publication selection for L-threonine.

2.2 General information

2.2.1 Chemistry

L- threonine, also named (2S,3R)-2-amino-3-hydroxybutyric acid, is an indispensable (essential), neutral amino acid. The molecular formula is $C_4H_9NO_3$. The CAS number for L-threonine is 72-19-5. The structural formula is shown in figure 2.2.1-1.

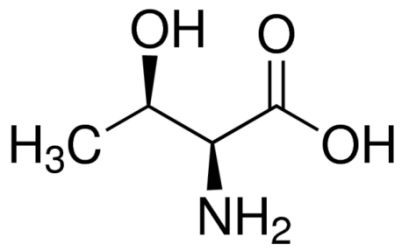

Figure 2.2.1-1: Structural formula of L-threonine.

2.2.2 Occurrence

All protein sources contain threonine. Animal and bean proteins have a slightly higher content (4-5% of total protein) than grain proteins (3-4% of total protein). Adequate amounts of threonine will be consumed when total protein intake meets recommendations, actually the threonine requirements (1050 mg/day for a 70 kg person, according to the WHO (2007) recommendation of 15 mg/kg bw per day) are certain to be exceeded several-fold.

2.3 Absorption, distribution, metabolism and excretion

2.3.1 In humans

Absorption

Free threonine from digested proteins is taken up from the small intestinal lumen by the sodium-amino acid cotransport systems B^o and ASC (Avissar et al., 2001; Munck and Munck, 1999). Threonine as a component of di-and tripeptides can also be taken up via hydrogen ion/peptide cotransporter 1 (SLC15A1, PepT1) and, to a lesser extent, hydrogen ion/peptide cotransporter 2 (SLC15A2, PepT2).

Export across the enterocyte basolateral membrane uses the sodium-amino acid cotransport systems A (ATA2) and ASC (ASCT1), and possibly other systems. As long as the amino acid concentration is higher inside the cell than in the basolateral space, transport can occur against the considerable sodium gradient. When the intracellular amino acid concentration falls below extracellular levels during fasting, transport turns towards the enterocyte and supplies needed amino acids.

Both the brush border membrane and the enterocyte basolateral membrane contain transporters that exchange amino acids. Neutral amino acids that are enriched in the cell by the sodium cotransporters are exchanged for a wide spectrum of other amino acids.

Metabolism

Threonine is needed for the synthesis of proteins, and practically all human proteins and peptides contain threonine. The metabolic pathway of L-threonine is closely linked to those of glycine and L-serine. L-threonine is not a substrate in transamination reactions. Threonine is mainly catabolised by threonine dehydratase to NH_3 and 2-ketobutyrate and further downstream to propionyl-CoA, which is rapidly and irreversibly converted to CO_2 in the Krebs cycle. An alternative, minor pathway (<10%) is catalysed initially by the enzyme threonine dehydrogenase before cleavage to form glycine and acetyl-CoA. By this latter pathway threonine will supply substrates for both the glucose and lipid synthesis (Darling et al., 2000). In energy terms, threonine yields 3.4 kcal/g.

Transport, cellular uptake and storage

Plasma concentration of threonine is about 130 µmol/l. It is lowest during the early morning hours and increases significantly after meals. Human studies have shown that threonine treatment has increased blood and cerebrospinal fluid levels of threonine but not of glycine (Hauser et al., 1992; Tsai and Huang, 1999). Uptake from the blood into tissues occurs via an array of transporters, many of them identical or similar to those mediating intestinal absorption. However, expression patterns are reported to vary greatly between different cell types.

The rise in cerebrospinal fluid threonine levels with treatment is in line with animal data indicating passage of this amino acid across the blood-brain barrier, but the specific roles of the different transport systems are not yet well understood.

Regarding feto-maternal transport, threonine is taken up from the blood across the brush border membrane of the syncytiotrophoblast by the sodium-amino acid cotransport system A (possibly also ASC and N), and the sodium-independent system L. Transfer across the basolateral membrane proceeds via the sodium-amino acid cotransport system A and the sodium-independent transporters EAT1 and LAT2.

Assuming an average threonine content of human proteins of 48 mg/g, a 70-kg man may have a mobilisable reserve of about 300 g.

Excretion

In the kidneys, free threonine filtered in the glomeruli is reabsorbed into the proximal renal tubuli by sodium-amino acid cotransport systems B^o and ASC. Dipeptides and tripeptides are taken up by via PepT1 and PepT2. The renal recovery of threonine is nearly complete in healthy prople. In normal gastrointestinal function, losses via faeces also are negligible. Most nitrogen from metabolised threonine is excreted into urine as urea. Minor metabolic products are aminoacetone and D-lactate, and the urinary excretion of these increases disprotionally with high threonine intake and in situations with low availability of free CoA.

2.4 Toxicological data/Adverse effects

2.4.1 Human studies

For the general human studies search, the first screening resulted in 3 full text articles, all included after full text reading, with the addition of one paper found by hand search. The search for studies in children and adolescents gave no titles that could be included after the first screening.

Table 2.4.1-1 An overview of human studies investigating adverse health effects of threonine supplementation.

Reference	Study design/ participant characteristics	Country	Number in treatment group		Dose	Main endpoint	Length of intervention	Adverse effects (verum groups)
			Active	Control				
RCTs (all with crossover design)								
Lee & Paterson 1993	Spinal spasticity patients, 33 patients (19 men and 14 women) 17 to 70 years	Northern Ireland	33	33	6 g/d	Spasticity	2 weeks	1 indigestion (moderate) 1 diarrhoea (moderate)
Hauser et al., 1992	Multiple sclerosis, 26 patients (11 men and 15 women), 41±6.5 years (mean±SEM)	USA	26	26	7.5 g/d	Spasticity	8 weeks	None (by diary)
Growdon et al., 1991	Familiar spastic paraparesis, 18 patients (14 male, 4 female) 23 – 70 years	USA	18	18	4.5 g/d (n= 8) 6 g/d (n=10)	Spasticity	2 weeks	None
Open, uncontrolled								
Barbeau et al., 1982	Genetic spasticity syndromes, 6 patients (4 women, 2 men), mean age 29.7 years	Canada	6	None	500 mg/d or more (up to 2.5 g/d)	Spasticity	1 year + 4 months observation	Dizziness (n=2) Nausea (n=3)

Lee and Paterson, 1993 (Northern Ireland)

A double-blind, placebo-controlled, cross-over, add-on design study of oral L-threonine at 6 g/day with 2-week treatment periods was performed in 33 patients with spinal spasticity (14 women, 19 men; age from 17 to 70 years) (Lee and Patterson, 1993). Multiple sclerosis was the diagnosis in 26 patients, spinal cord injury in five, syringomyelia in one, and spinal tumour in one. Fourteen patients were on no antispastic therapy during the trial, 12 were on baclofen alone, and 7 on a combination of baclofen and either dantrolene or diazepam. Patients were recruited from neurology and spinal injury clinics and from hospital wards for the young disabled.

There were four study periods: 1) four-week baseline, 2) two-week treatment period, 3) two-week washout, and 4) two-week treatment period. L-threonine 6 g/day was given as 500 mg capsules three times daily on an empty stomach; placebo capsules had the same appearance and taste. Treatment randomisation was carried out by a medical statistician and the codes were held by the hospital pharmacy. Blocks of six were used in the random number generation. Patients' and caregivers' opinions on improvement were recorded and any side effect documented.

There was a strong rise in plasma threonine levels during therapy. Four patients who started the study failed to complete it because of intercurrent infections (n=2) or non-medical

reasons (n=2). Two patients on L-threonine treatment reported minor side-effects which did not prevent them from completing the study: indigestion and diarrhoea were each reported once. One patient complained on headaches on placebo treatment.

Hauser et al., 1992 (USA)

Twenty-six patients (15 females, 11 men; mean (±SEM) age 41±6.5 years) with clinically definite multiple sclerosis were enrolled in this double blind, placebo-controlled cross-over study (Hauser et al., 1992). Ambulatory patients with inactive or very slowly progressive multiple sclerosis were eligible (definitions of 'inactive' and 'very slowly progressive' as well as inclusion and exclusion criteria are specified). Threonine was supplied as a purified amino acid in 500-mg capsules prepared by the hospital pharmacy. Active and placebo capsules (lactose) vere identical in appearance and taste. Group assignment was performed by the hospital pharmacist according to a prescheduled randomised computer-generated order of administration. The protocol consisted of two eight-week treatment periods separated by a two-week washout period. During each treatment period, the patients were asked to self-administer five capsules three times daily, for a total daily dose of 7.5 g. Compliance was assessed by frequent patient contact, by return of empty capsule bottles, and by measurement of serum threonine levels. Before treatment, patients were instructed by a research dietitian to consume a standard 75-g protein diet, to be continued until completion of the study. Patients were asked to keep a diary form on which to record clinical symptoms of spasticity, as well as any side effects or illnesses they experienced. The treatment code was broken after all clinical and laboratory data had been entered into the database. Twenty-four patients completed at least one treatment period and 21 completed both treatment periods. Two patients dropped out during each treatment period for specified reasons, none because of treatment side effects. No side effects or toxic effects of threonine were identified.

Growdon et al., 1991 (USA)

According to a double-blind, crossover protocol with 2-week intervention periods, L-threonine doses of 4.5 and 6.0 g/day were given to 18 patients (4 female, 14 male; age range 23 to 70 years) with familial spastic paraparesis (Growdon et al., 1991). Threonine was given in 500 mg capsules prepared by the institution's research pharmacy; seven patients took 1.5 g three times daily (total 4.5 g/day) and nine patients took 2.0 g three times daily (total 6.0 g/day); one patient took both doses and completed the study twice. Placebo capsules contained lactose and were identical to threonine capsules in appearance, texture and taste when intact. The response to treatment was evaluated at the end of each 2-week period. Blood specimens and cerebrospinal fluid samples were collected for each period. Plasma and cerebrospinal fluid threonine levels rose significantly during treatment.There were no noticeable clinical side effects of L-threonine administration (method of registration not indicated); the results of hematologic, hepatic and renal function tests did not change during treatment.

Barbeau et al., 1982 (Canada)

This is a one-year pilot study in which threonine supplementation (500 mg/day for most of the intervention period) was given to six patients (four women, two men; mean age 29.7 years) with genetic spasticity syndromes for an overall period of 12 months, followed by a four month observation period without supplementation (Barbeau et al., 1982). Threonine was given in gelatine capsules of 250 mg. In detail, the protocol included an initial three-week stay in the hospital metabolic unit, during which the threonine dose was increased gradually to tolerance, or to a maximum dose of 2.5 g/day. Upon discharge from the hospital, the dose of threonine was gradually decreased over the next two months to 500 mg/day in most patients (range 500-1000 mg/day) and maintained at this dose for 9 months. One year after intervention start threonine supplementation was stopped, and the patients were observed for a further four months without any treatment.

Two patients experienced episodes of dizziness ("giddiness") in the early phase of treatment, and three suffered from occasional nausea. No other symptoms could be associated with the treatment (method of registration not indicated). Hematological and clinical chemistry parameters (SMA-15) remained unchanged throughout the treatment.

2.4.1.1 Interactions

There was no information concerning interactions in the literature reviewed in the present risk assessment, apart from normal biochemical interactions during threonine metabolism. The absence of information in the selected literature does not document an absence of adverse interactions.

2.4.1.2 Allergic sensitisation (including adjuvant effects)

There was no information concerning allergic sensitisation or allergy adjuvant effects in the literature reviewed in the present risk assessment. Threonine per se would not be expected to behave as an allergen. The absence of information in the selected literature does not document an absence of allergic sensitisation or allergy adjuvant effects.

2.4.2 Animal studies

Of the titles identified in the search for animal studies, one study was included after the first screening and full text reading. This study is summarised in Table 2.4.2-1.

Table 2.4.2-1: Overview of included animal studies investigating L-threonine 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** (verum group)	**NOAEL (mg/kg bw/day)**
van de Mortel et al. (2010)	Sprague-Dawley rats, 10 rats per group per sex, randomised	Threonine N-acetyl threonine	Standard rodent diet, supple-mented to give 1000 mg/kg bw/day of threonine or 100, 500, and 1000 mg/kg bw/day of N-acetyl-threonine + untreated control group	OECD guide-line 407 (plus muta-genicity and acute toxicity)	4-week dietary study	No biologically significant adverse effects neither for threonine nor for N-acetyl-threonine	M: 854.3 F: 932.2

Safety assessment of N-acetyl-L-threonine (van de Mortel et al., 2010)

The purpose of this study was to investigate whether 4-week intake of a N-acetyl-L-threonine supplemented diet would show any adverse effects in the Sprague-Dawley rat (both sexes) as determined according to the OECD Guideline 407 test protocol(van de Mortel et al., 2010). A group administered threonine 1000 mg/kg bw per day (dose aim) incorporated into a standard rodent diet was included as a control group in addition to untreated controls. All rats in the study survived until scheduled sacrifice, and no biologically significant differences from the normal controls were observed in any group for body weight, feed consumption, clinical signs, behavioural, ophthalmology, hematology, coagulation, clinical chemistry, organ weights or gross or microscopic changes. Consumed doses of threonine were 854.3 mg/kg bw per day for males and 932.2 mg/kg bw per day for females. Thus, the NOAEL for threonine demonstrated in this study can be considered to be the dose tested in males, 854.3 mg/kg bw per day. This finding is supported by the demonstration of a NOAEL of 848.5 mg/kg bw per day for N-acetyl-L-threonine which has been demonstrated to have a biological availability close to that of L-threonine (Boggs, 1978).

2.4.3 *In vitro* studies

The available data do not give rise to safety concern with respect to genotoxicity (EFSA, 2006; van de Mortel et al., 2010).

2.4.4 Mode of action for adverse effects

No specific or definite mechanisms for any potential adverse effects have been described. However, dietary threonine imbalance is known to reduce food intake and weight gain and the growth of the small intestine, liver, and skeletal muscle in young animals. Still, when added to low-protein diets, threonine causes less growth depression than other amino acids (Garlick, 2004). There were data suggesting increased muscle protein breakdown in weanling rats given diets containing excess amounts (0.5%-4%) of threonine (Sarwar et al., 1995). In a study by Wang et al. (2007) in a 25 day-old pig model, protein synthesis in skeletal muscle as well as jejunal mucosa and mucins was reduced to a greater extent than in the liver in response to an imbalance of dietary threonine (150% of recommended feed levels). These results suggest that an excess of dietary threonine decreases protein synthesis in rapidly growing tissues of young pigs. The findings provide an explanation for the lower growth performance of animals fed a threonine-imbalanced diet, although the specific underlying biochemical mechanism is largely unknown.

2.4.5 Vulnerable groups

No vulnerable groups to excess doses of L-threonine have been reported, apart from suggestions from animal studies that excess threonine may reduce protein synthesis in rapidly growing tissues of very young animals. However, threonine has not been studied extensively but appears to be one of the least toxic of the amino acids (Garlick, 2004; Peng et al., 1973).

There have been no reported studies involving children, elderly, pregnant women or lactating women.

2.5 Summary of hazard identification and characterisation

The information provided in previous risk assessment supports the notion that L-threonine has low, if any, toxicity, and no specific safety concerns have been raised. No studies, however were identified that provide data to support the determination of specific safe dose levels, apart from the AESAN (2012) approval of a dose of 1150 mg/day.

For the risk evaluation of specified doses of L-threonine in food supplements, literature searches including both human and animal studies were performed. In these searches, we did not find any long-term studies in healthy individuals that could be used for safety evaluation. One small (n=6) uncontrolled pilot study of one year's duration and three randomised crossover trials of eight, two, and two weeks duration, respectively, in

individuals suffering from muscular spasticity disorders of different ethiologies were identified. These studies were considered relevant for the present risk evaluation, because the disorders of the patients included in the studies are not likely to have significantly affected threonine absorption, distribution, and metabolism. Additionally, one four-week toxicity study in rats performed according to OECD guideline 407 was retrieved.

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

1. No long-term studies on L-threonine in healthy children, adolescents or adult humans were found. A small (n=6), open one-year pilot study in patients with spastic disorders treated with doses ranging from 0.5 to 2.5 g/day was found but can only serve as a supportive study.
2. No adverse effects (diary method of registration) were reported in one randomised placebo controlled eight-week clinical trial with 7.5 g/day of threonine treatment.
3. No adverse effects apart from one case of indigestion and one case of diarrhoea were observed in two randomised placebo controlled trials of two weeks' duration (n=33, n=18) in which 6 g/day respective 4.5 g/day of L-threonine was given.
4. In the literature, L-threonine is generally considered to be one of the amino acids with the least potential for toxicity, and produces innocuous metabolites.
5. There are some indications from animal studies that excess doses of threonine may have an adverse effect on protein synthesis in rapidly growing tissues in young animals.
6. In rats, a 28-day subchronic toxicity study was identified, with a NOAEL of 854.3 mg/kg bw per day in males and 932.2 mg/kg bw per day in females. No adverse health effects were reported at the only dose tested. - The NOAEL in male rats corresponds to 59.8 g/day in a 70 kg human.

For the risk characterisation of L-threonine, in the absence of long-term human studies in healthy individuals, VKM will base the value of comparison on the NOAEL demonstrated in a 8-week randomised placebo controlled study in humans, that is 7500 mg corresponding to 107 mg/kg bw per day.

3 Exposure / Intake

Exposure of L-threonine was 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 1000, 1200, 1500, 2000 and 2400 mg/day of L-threonine 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-threonine from specified doses in food supplements in children, adolescents and adults.

Groups	Daily doses (mg)	Body weight (kg)	Exposures (mg/kg bw per day)
Children (10 to <14 years)	1000, 1200, 1500, 2000 and 2400	43	23, 28, 35, 46 and 55
Adolescents (14 to <18 years)	1000, 1200, 1500, 2000 and 2400	61	16, 20, 25, 33 and 39
Adults (≥18 years)	1000, 1200, 1500, 2000 and 2400	70	14, 17, 21, 29 and 34

3.2 Other sources

Threonine is an essential amino acid, with no endogenous synthesis. In the normal diet, the amino acids are ingested as components of food proteins and not as free acids. The 2007 WHO recommendation is 15 mg/kg bw per day corresponding to 1050 mg/day for a 70-kg person. Deducing the amount of individual amino acids from soy bean protein, an intake of 100 g protein, which is not an unusual intake for an adult European individual, would amount to an intake of 4 g threonine or 57 mg/kg bw per day for a 70-kg person.

Based on NHANES III (1988-1994), the overall mean intake of L-threonine from food and food supplements in the United States was 3.0 g/day (43 mg/kg bw per day for a 70-kg person). Men aged 51-70 years had the highest intakes at the 99th percentile of 7.1 g/day (IOM, 2005).

4 Risk characterisation

The doses received from NFSA for assessment were 1000, 1200, 1500, 2000 and 2400 mg/day L-threonine in food supplements, and the estimated exposures for adults, adolescents and children 10 years and older derived from these dose levels are given in chapter 3.

The value used for comparison with the estimated exposure in the risk characterisation is the NOAEL defined in a 8-week randomised placebo controlled study in humans, 7500 mg/day, with diary registration of adverse events. For a 70-kg individual, this corresponds to 107 mg/kg bw per day.

The NOAEL defined in a 4-week toxicity study in rodents, 854.3 mg/kg bw per day was based on the only dose tested, and a LOAEL has not been determined.

Our literature review did not reveal any studies of threonine in children or adolescents, and there were no studies in children 10 years or older included in previous risk assessments. There are animal data suggesting that very young individuals are more vulnerable than adults for excessive doses of threonine, with reduced protein synthesis in fast-growing tissues as the adverse outcome. However, with an intake in the age groups considered in this risk evaluation in the form of supplements in amounts similar to or lower than what is obtained from food by adult individuals, it is considered to be unlikely that protein synthesis will be affected from the doses of threonine under evaluation. No tolerance level is therefore set for threonine specifically for children or adolescents. Assuming similar tolerance for these age groups as for adults, the same value for comparison as for adults is used for children and adolescents (107 mg/kg bw per day).

Table 4-1: The calculated margins between the highest dose tested in humans not associated with adverse effect (107 mg/kg bw per day) and the exposure to L-threonine from food supplements (MOE-values) for the various age groups.

Age groups	1000 mg/day	1200 mg/day	1500 mg/day	2000 mg/day	2400 mg/day
Children (10 to <14 years) (43.4 kg)	5	4	3	2	2
Adolescents (14 to <18 years) (61.3 kg)	7	4	3	3	3
Adults (≥18 years) (70 kg)	7	6	5	4	3

The calculated MOE-values for data from the human study ranged from 2 to 7 (Table 4-1) for a daily intake of 1000-2400 mg/day of L-threonine.

MOE-values below 10 (for interindividual differences in humans) were regarded as acceptable since L-threonine is a nutrient that does not cause any known adverse health effects. In addition, the overall mean threonine intake according to NHANES III (3 g/day) is larger than the doses considered in the present risk assessment.

VKM considers that:

In adults (≥18 years), the specified doses 1000, 1200, 1500, 2000 and 2400 mg/day L-threonine in food supplements are unlikely to cause adverse health effects.

In adolescents (14 to <18 years), the specified doses 1000, 1200, 1500, 2000 and 2400 mg/day L-threonine in food supplements are unlikely to cause adverse health effects.

In children (10 to <14 years), the specified doses 1000, 1200, 1500, 2000 and 2400 mg/day L-threonine in food supplements are unlikely to cause adverse health effects.

5 Uncertainties

- Long-term safety in healthy adults, adolescents and children has been extrapolated from shorter studies in adults
- Toxicity in rodents according to OECD guidelines has been determined only in a one-dose-level, four-week study in the rat (OECD Guideline 407)
- Whether reduced protein synthesis with large intakes of threonine as observed in very young animals may be relevant for the youngest age group in our risk assessment
- The human studies in our risk assessment include subjects suffering from multiple sclerosis, a condition supposed not to significantly affect threonine metabolism and the risk of adverse effects. However, a recent publication (Negrotto and Correale, 2017) suggests that amino acid catabolism in multiple sclerosis may be decreased compared to what is observed in healthy individuals. The consequences, if any, for amino acid tolerance remain to be determined

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-threonine in food supplements at the doses 1000, 1200, 1500, 2000 and 2400 mg/day for the general population, ages 10 years and above. The literature searches were conducted 5 April, 16 September and 13 October 2016, and included both human studies and animal studies.

The literature search did not reveal any relevant studies in children (10 to <14 years) and adolescents (14 to <18 years). No data have been found indicating that children in this age group or adolescents are more vulnerable than adults for relevant doses of L-threonine and no tolerance level is set for L-threonine specifically for children or adolescents. The conclusions are therefore based on the assumption of similar tolerance for children and adolescents as for adults.

Four human studies (two two-week studies, one eight-week study and a small one-year pilot study) and one four-week animal toxicity study are included in this report. For the risk characterisation of L-threonine, in the absence of long-term human studies in healthy individuals, VKM bases the value of comparison on the highest dose tested in one 8-week study in humans. The value of comparison is set to 107 mg/kg bw per day, corresponding to 7500 mg/day in a 70 kg adult. The NOAEL determined in the four-week subchronic rat study supports the suggestion that these doses will be well tolerated in humans, and so do the two human two-week studies and the pilot study. MOE-values from 2 to 7 were regarded as acceptable since L-threonine is a nutrient that does not cause any known adverse health effects. In addition, the overall mean threonine intake according to NHANES III (3 g/day) is slightly larger than the doses considered in the present risk assessment. The requirement for threonine, 15 mg/kg bw/day, corresponding to– 1.1 g/day, is of similar magnitude as the doses considered in the present risk assessment.

No particular vulnerable groups for L-threonine supplements have been identified.

VKM concludes that:

In adults (≥18 years), the specified doses 1000, 1200, 1500, 2000, and 2400 mg/day L-threonine in food supplements are unlikely to cause adverse health effects.

In adolescents (14 to <18 years), the specified doses 1000, 1200, 1500, 2000, and 2400 mg/day L-threonine in food supplements are unlikely to cause adverse health effects.

In children (10 to <14 years), the specified doses 1000, 1200, 1500, 2000, and 2400 mg/day L-threonine 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-threonine in food supplements. Green: Estimated exposures to L-threonine are unlikely to cause adverse health effects.

	L-threonine				
Doses Age groups	1000 mg/day	1200 mg/day	1500 mg/day	2000 mg/day	2400 mg/day
Children (10 to <14 years)					
Adolescents (14 to <18 years)					
Adults (≥18 years)					

7 Data gaps

- There is a lack of data from long-term human studies investigating potential adverse effects from L-threonine supplementation
- No studies are found that include effects of threonine supplementation in lactating or pregnant women
- No adequate animal studies on chronic toxicity, metabolic disturbances and carcinogenicity are available

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Appendix 1

Search strategies for this risk assessment

Search strategy human studies

Database: Embase <1974 to 2016 April 05>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present>

1. threonine*.ti. (9191)
2. (risk* or safety or adverse or side-effect*1 or hazard* or harm* or negative or contraindicat* or
3. contra-indicat* or interact* or toxicity or toxic).tw. (9789711)
4. 1 and 2 (1781)
5. (conference abstract* or letter* or editorial*).pt. (4934471)
6. 3 not 4 (1709)
7. limit 5 to (danish or english or norwegian or swedish) (1677)
8. limit 6 to human (679)
9. remove duplicates from 7 (427)

Search strategy studies in children and adolescents

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

1. threonine*.ti. (9246)
2. (child* or adolescent* or teenage* or college* or high school).tw. (3093526)
3. 1 and 2 (24)
4. limit 3 to (danish or english or norwegian or swedish) (23)
5. remove duplicates from 4 (17)

Search strategy animal studies

Database: Embase <1974 to 2016 September 16>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present>

1. threonine*.ti. (9359)
2. (risk* or safety or adverse or side-effect*1 or hazard* or harm* or negative or contraindicat* or contra-indicat* or interact* or toxicity or toxic).tw. (10266610)
3. 1 and 2 (1832)
4. (conference abstract* or letter* or editorial*).pt. (5175652)
5. 3 not 4 (1758)

6. limit 5 to (danish or english or norwegian or swedish) (1726)
7. limit 6 to animals (609)
8. remove duplicates from 7 (440)