Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

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Risk assessment of "other substances" – L-tryptophan

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

Report from the Norwegian Scientific Committee for Food Safety (VKM) 2016: 04
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Assessed and approved

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

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

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Competence of VKM experts

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

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Summary

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

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

The present report is a risk assessment of L-tryptophan and is based on previous risk assessments of L-tryptophan and scientific papers retrieved from systematic literature searches.

L-tryptophan is an indispensable amino acid in humans, which in addition to its role in protein synthesis, also participates in complex metabolic pathways where it acts as a precursor to the potent neurotransmitter serotonin, the hormone melatonin, and the vitamin niacin (vitamin B_3). L-tryptophan is available from a wide variety of protein-rich foods in the normal diet, including meat, fish, milk and dairy products, egg, beans, lentils and also bread and grains, pasta, rice, fruit and vegetables.

According to information from NFSA, L-tryptophan is an ingredient in food supplements sold in Norway. NFSA has requested a risk assessment of the following doses of L-tryptophan in food supplements: 250 mg/day, 300 mg/day, and 450 mg/day for adults, adolescents and children 10 years and above. Usual dietary intake of L-tryptophan in Norway is not known, but data from the USA and the UK suggest an average dietary intake of about 900 mg/day of which the main part is bound in food protein.

In phase 1 we have identified seven previous reports that have aimed to assess the safety of L-tryptophan supplementation in humans; the most recent was published by VKM in 2013. To complement the existing reports, a literature search was performed in MEDLINE and EMBASE to retrieve studies published in the period 2012-2015. This search retrieved two recent randomised trials with L-tryptophan. In addition, we performed a literature search concerning safety of L-tryptophan in children and adolescents with no time restriction. This search retrieved no relevant results that met the inclusion criteria.

Four aspects related to safety of L-tryptophan were identified in previous reports: 1) adverse effects reported at high doses, including appetite suppression, nausea and vomiting, faintness, dizziness, drowsiness, tremor, fatigue, and headache; 2) a suggested, but not

established, increased risk of cataract; 3) the eosinophilia-myalgia syndrome (EMS), which is thought to be caused by contaminants produced in the manufacturing of L-tryptophan supplements, however this is still unresolved; 4) the risk of adverse drug reactions caused by excessive serotonergic action by concomitant use of antidepressants, including monoamine oxidase inhibitors, selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, tricyclic antidepressants and other drugs, known as the *serotonin syndrome*.

According to previous reports, doses of 3 to 6 g/day of L-tryptophan have been associated with adverse effects. Using an uncertainty factor of 10, conclusions in previous reports have suggested a maximum level of 220 mg/day for adults. An upper tolerable intake level (UL) of 220 mg/day was first proposed in a report by the UK Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) in 2004, and was derived from the average dose of L-tryptophan consumed as a prescription drug against depression in the UK at the time (2228 mg/day). This level has been maintained in later reports by other committees, most recently VKM in 2013 as a tentative guidance level. Additional information from the publications retrieved in the literature search did not provide evidence of sufficient weight to change the previous conclusions concerning UL.

There is a lack of well-designed supplementation studies with L-tryptophan in humans designed to address adverse effects and dose-response relationship as primary outcome. There is also a lack of data about potential adverse health effects of L-tryptophan supplementation in children and adolescents.

Patients using antidepressant drugs constitute a specific vulnerable subgroup of the population with regard to possible adverse effects of L-tryptophan supplements, due to the potentially life-threatening drug interaction effects that occur from excessive serotonergic action.

The Norwegian Scientific Committee for Food Safety (VKM) concludes that:

- In adults (≥18 years), the specified doses 250, 300, and 450 mg/day L-tryptophan in food supplements may represent a risk of adverse health effects.
- In adolescents (14 to <18 years), the specified doses 250, 300, and 450 mg/day L-tryptophan in food supplements may represent a risk of adverse health effects.
- In children (10 to <14 years), the specified doses 250, 300, and 450 mg/day L-tryptophan in food supplements may represent a risk of adverse health effects.

Children below 10 years were not included in this assessment.

Short summary:

The Norwegian Scientific Committee for Food Safety (VKM) has, at the request of the Norwegian Food Safety Authority, assessed the risk of specified doses of L-tryptophan in food supplements. VKM concludes that the specified doses 250 mg/day, 300 mg/day, and

450 mg/day tryptophan may represent a risk of adverse health effects in adults (≥18 years), adolescents (14 to <18 years), and children (10 to <14 years).

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

Sammendrag på norsk

På oppdrag for Mattilsynet har Vitenskapskomiteen for mattrygghet (VKM) vurdert risiko ved tilsetting av "andre stoffer" i kosttilskudd og energidrikker som selges i Norge, i henhold til spesifikke bruksdoser oppgitt fra Mattilsynet. Disse risikovurderingene vil gi Mattilsynet et 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 risiko ved aminosyren L-tryptofan. Risikovurderingen er basert på tidligere risikovurderinger av aminosyren og vitenskapelige artikler som er funnet i systematiske litteratursøk.

L-tryptofan er en essensiell aminosyre for mennesker. I tillegg til dens rolle i proteinsyntese, inngår tryptofan i komplekse metabolske omsetningsveier hvor den er en forløper til nevrotransmitteren serotonin, hormonet melatonin og vitaminet niacin (vitamin B_3). I et vanlig kosthold finnes L-tryptofan i et bredt utvalg proteinrike matvarer som kjøtt, fisk, melk og meieriprodukter, egg, bønner, linser, samt brød og kornprodukter, pasta, ris, frukt og grønnsaker.

Ifølge informasjon fra Mattilsynet er L-tryptofan en ingrediens i kosttilskudd som selges i Norge. Mattilsynet har bedt om en risikovurdering av følgende doser i kosttilskudd: 250, 300 og 450 mg/dag for voksne, ungdom og barn 10 år og eldre. Inntak fra kosten i Norge er ikke kjent, men data fra USA og Storbritannia viser et gjennomsnittlig daglig inntak på om lag 900 mg/dag.

I fase 1 identifiserte vi 7 tidligere rapporter som hadde som formål å vurdere risiko ved tryptofantilskudd til mennesker. Den nyeste ble publisert av VKM i 2013. For å komplettere de eksisterende rapporter ble det gjennomført et litteratursøk i MEDLINE og EMBASE for å fange opp eventuelle studier publisert i 2012-2015. Her ble to randomiserte studier som ga tilskudd av L-tryptofan inkludert. I tillegg ble det gjort et litteratursøk vedrørende negative helseeffekter av tryptofan blant barn og unge, uten tidsbegrensning. Dette søket fanget ikke opp noen relevante publikasjoner som oppfylte inklusjonskriteriene.

Fire aspekter vedrørende trygghet av tryptofantilskudd er identifisert i tidligere rapporter: 1) ubehagelige bivirkninger ved høye doser, som manglende appetitt, kvalme og oppkast, svimmelhet, søvnighet, skjelving, utmattelse og hodepine; 2) mistanke om økt risiko for katarakt (grå stær); 3) eosinofili-myalgi-syndromet (EMS), som antas å være forårsaket av forurensninger produsert i fremstilling av tryptofantilskudd, selv om dette fremdeles er uavklart; 3) risiko for bivirkninger forårsaket av forhøyet serotonerg aktivitet ved samtidig bruk av antidepressive legemidler, inkludert monoamino-oksidasehemmere, selektive

serotoninreopptakshemmere, selektive serotonin- og noradrenalinreopptakshemmere, trisykliske antidepressiver m.m., kjent som "serotonin-syndromet".

Ifølge tidligere rapporter har doser mellom 3 og 6 g/dag vært forbundet med negative helseeffekter. Ved å benytte en usikkerhetsfaktor på 10, har konklusjonene i de tidligere rapporter antydet et øvre inntaksnivå på for voksne på 220 mg/dag. Dette ble først definert som et tolerabelt øvre inntaksnivå av Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) i Storbritannia i 2004, basert på gjennomsnittsdosen av tryptofan brukt som reseptbelagt legemiddel (antidepressivum) i Storbritannia på den tiden (2228 mg/dag). Dette øvre nivået har vært opprettholdt i senere rapporter av andre komitéer, senest som et tentativt veiledende nivå i VKMs risikovurdering publisert i 2013. Ytterligere informasjon fanget opp i litteratursøket ble ikke ansett for vektig nok til å ha noen innflytelse på konklusjonene i det eksisterende kunnskapsgrunnlaget.

Det er mangel på veldesignede supplementeringsstudier som har som primært formål å undersøke potensielle negative helseeffekter av tryptofan og dose-respons-sammenheng mellom tryptofan og negative helseeffekter hos mennesker. Det er også mangel på studier som har undersøkt mulige negative helseeffekter av tryptofan hos barn og unge.

Pasienter som bruker antidepressive legemidler utgjør en spesielt sårbar gruppe med hensyn til mulige negative helseeffekter av tilskudd med L-tryptofan, på grunn av de potensielt livstruende interaksjonseffekter som kan oppstå ved forhøyet serotonerg aktivitet.

Vitenskapskomiteen for mattrygghet (VKM) konkluderer med at:

- For voksne (≥18 år) vil dosene 250, 300 og 450 mg/dag L-tryptofan i kosttilskudd kunne representere en risiko for negative helseeffekter.
- For ungdom (14 til <18 år) vil dosene 250, 300 og 450 mg/dag L-tryptofan i kosttilskudd kunne representere en risiko for negative helseeffekter.
- For barn (10 til <14 år) vil dosene 250, 300 og 450 mg/dag L-tryptofan i kosttilskudd kunne representere en risiko for negative helseeffekter.

Barn under 10 år inngår ikke i oppdraget.

Kort sammendrag:

Vitenskapskomiteen for mattrygghet (VKM) har på oppdrag fra Mattilsynet vurdert risiko ved inntak av spesifikke doser av aminosyren L-tryptofan i kosttilskudd. VKM konkluderer med at dosene 250 mg/dag, 300 mg/dag og 450 mg/dag L-tryptofan i kosttilskudd vil kunne representere en risiko for negative helseeffekter hos voksne (≥18 år), ungdom (14 til <18 år), og barn (10 til <14 år).

Abbreviations and glossary

Abbreviations

5-HIAA - 5-hydroxyindoleacetic acid, the main metabolite of serotonin
5-HT - 5-hydroxytryptamine (serotonin)
AESAN - Spanish Agency for Food Safety and Nutrition
AFSSA - French Food Safety Agency (up to 1st July 2010)
ANSES - French Agency for Food, Environmental and Occupational Health and Safety (from 1st July 2010)
bw - body weight
COT - The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (UK)
CNS - central nervous system
EBT - 1,1'-ethylidene-bis-L-tryptophan
EFSA - European Food Safety Authority
EMS - eosinophilia-myalgia syndrome
GI - gastrointestinal
IOM - Institute of Medicine, USA
LD_{50} - lethal dose for 50% of the animals
LNAA - large neutral amino acids: tyrosine, phenylalanine, leucine, isoleucine, valine
LOAEL - lowest observed adverse effect level
MAO - monoamine oxidase
MAOI - monoamine oxidase inhibitors
NFSA - Norwegian Food Safety Authority [*Norw.*: Mattilsynet]
NOAEL - no observed adverse effect level
POMS - profile of mood states questionnaire
RCT - randomised controlled trial
SNRI - serotonin-norepinephrine reuptake inhibitors
SSRI - selective serotonin reuptake inhibitors
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 and WHO has established the following definition for "adverse effect": a change in morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences (WHO, 1994).

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-tryptophan in food supplements at the following doses: 250 mg/day, 300 mg/day, and 450 mg/day.

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

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

Assessment

1 Introduction

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

VKM has in this series of risk assessments of "other substances" not evaluated any claimed beneficial effects from these substances, but merely possible adverse effects at specified doses used in Norway. This risk assessment regards the substance L-tryptophan per se, and no specific products.

According to information from the Norwegian Food Safety Authority (NFSA), L-tryptophan is an ingredient in food supplements purchased in Norway. NFSA has requested a risk assessment of the following doses of L-tryptophan from food supplements: 250 mg/day, 300 mg/day, and 450 mg/day.

L-tryptophan is an essential amino acid bound to and available from most food proteins in the normal diet. The average daily requirement in humans is well defined: 4 mg/kg bw per day in adults, 4.5 mg/kg bw per day in 15-18-year-olds, and 4.8 mg/kg bw per day in 11-14-year-olds (EFSA, 2012).

Besides its proteogenic role, L-tryptophan is a precursor for small molecules with important functions, including the neurotransmitter serotonin (5-hydroxytryptamine) which regulates mood, appetite and sleep, and in turn the pineal hormone melatonin which regulates circadian rhythm and sleep. Through a different metabolic pathway L-tryptophan also serves as the precursor for niacin (vitamin B_3) which participates in energy metabolism, sterol hormone synthesis and DNA repair (see section 2.3).

L-tryptophan in doses of 500 mg/day and higher is available in some countries as a prescription drug for treatment of depression (Anatomical Therapeutic Classification code: N06A X02).

Information on habitual dietary intake of L-tryptophan in Norway is not available. In a nationwide US dietary study (NHANES III 1988-1994), mean dietary intake of L-tryptophan was 0.9 g/day. In the age groups with the highest intake, the 99-percentile was 2.1 g/day in men aged 51-70 years, and 1.3 g/day in women aged 19-30 years (IOM, 2005).

2 Hazard identification and characterisation

2.1 Literature

The present risk assessment is based on previous risk assessments of L-tryptophan and scientific papers retrieved from a comprehensive literature search.

2.1.1 Previous risk assessments

Risks related to L-tryptophan have previously been evaluated by the Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment (COT), United Kingdom, 2004; the Institute of Medicine (IOM), USA, 2005; the French Food Safety Agency (AFSSA), France, 2009 and 2011; the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN), Spain, 2012; and the Norwegian Scientific Committee for Food Safety (VKM), Norway, 2011 and 2013. The results of these reports in terms of dose-response assessment of L-tryptophan are summarised in Table 2.1.1-1, and more detailed information is given in the text below.

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

Risk assessment body, country and publication year	Dose-response assessment
COT (2004), UK	NOAEL: 2228 mg/day UL: 220 mg/day
IOM (2005), USA	Not established
AFSSA (2009), France	LOAEL: 3000 mg/day UL: 220 mg/day
ANSES (2011), France	Not established
VKM (2011), Norway	Not established
AESAN (2012), Spain	UL: 300 mg/day
VKM (2013), Norway	UL: not established. Tentative guidance level (GL): 220 mg/day

COT Statement on Tryptophan and the Eosinophilia-Myalgia Syndrome. 2004, United Kingdom, 2004

The background for this statement was the 1989 outbreak of the so-called eosinophilia-myalgia syndrome (EMS) associated with the consumption of L-tryptophan-containing dietary supplements. EMS is a reversible, but potentially life-threatening multisystem disorder, characterised by raised blood concentration of eosinophils (>1.0 x 10^9/L) and severe myalgia, often accompanied by arthralgia, dyspnea, fever, neuropathy, peripheral oedema

and skin lesions which can include sclerosis or papular and urticarial lesions. Cases of EMS were reported in the US, the UK, Germany, Canada, Belgium, France, Israel and Japan. In epidemiological studies, 97-100% of EMS cases were traced to a certain L-tryptophan supplement manufactured by the company Showa Denko in Japan. This supplement was produced by a fermentation process involving the use of the bacterium *Bacillus amyloliquefaciens*, and resulted in increased levels of intermediate products in L-tryptophan synthesis. Significant associations were shown between the batches of L-tryptophan associated with EMS and the use of this bacterial strain (COT, 2004).

More than 60 contaminants were identified in the EMS-associated batches. When comparing batches of EMS-associated L-tryptophan, non-EMS associated L-tryptophan from the same manufacturer, and control L-tryptophan from another manufacturer, six of these contaminants were associated with EMS. At the time of the report, five of the contaminants had been identified and one remained unidentified. Toxicological studies have been performed, supporting that the cases are likely to be due to the effects of contaminants.

In particular, one of the contaminants, 1,1'-ethylidene-bis-L-tryptophan (EBT) has been identified as the possible causal agent. This substance has been associated with haematological changes, necrosis, and inflammation of the fascia and perimysium in animal studies.

At the time the report was published, L-tryptophan was available as a prescription drug in the UK for the treatment of depression unresponsive to other antidepressants (Anatomical Therapeutic Classification code: N06A X02).

The Committee stated as follows: "Only one product is currently available in the UK, the Merck Pharmaceuticals product Optimax. At the time of the EMS epidemic four possible cases of EMS were linked to Optimax. One of the cases was confirmed as matching all the CDC criteria for EMS. It has been suggested that Optimax may not have been the only source of L-tryptophan this case had been exposed to. Because of the concern over EMS, a unit was established by the manufacturers in 1994 to facilitate the monitoring of patients prescribed Optimax. Prescribers and patients must be registered with the unit in order to receive Optimax. Prescribers are sent an initial questionnaire on registration, followed by questionnaires at 3 and 6 months and thereafter every 6 months. Since monitoring began over 5000 patients have been treated with Optimax, with a mean dose of 2228 mg/day L-tryptophan. The manufacturers stated that up-to-date questionnaires are available for 96% of patients. No patients meeting all the CDC criteria for EMS have been identified."

Thus, based on information from the pharmaceutical company, the COT concluded that as a prescription drug, L-tryptophan had not resulted in a detectable increase in EMS risk. Applying an uncertainty factor of 10 to the mean therapeutic dose of 2228 mg tryptophan per day, to allow for uncertainty with respect to the actual cause of EMS, indicated that a dose of 220 mg tryptophan per day as a dietary supplement would not present an appreciable risk to health, providing that it meets the purity criteria specified in the European Pharmacopoeia.

In December 2005 (18 months after publication), the COT agreed that the statement should be amended to state that the mean therapeutic dose referred to in the conclusion was without adverse effect and so represented a no observed adverse effect level (NOAEL).

It was not within the scope of the COT statement to consider any other potential adverse effects of L-tryptophan supplementation beyond EMS. No systematic literature search was described, and no randomised controlled trials with L-tryptophan supplementation were included in the bibliography of the COT report.

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

The main focus of this extensive report was to establish dietary reference intakes for amino acids and various other nutrients. Potential toxicity of tryptophan was discussed briefly. Both animal studies (see section 2.4.2) and human studies were considered. The committee concluded that taken together, the available human studies indicate that relatively short-term (acute and subacute) use of L-tryptophan is associated with appetite suppression, nausea, and drowsiness (IOM, 2005). However, in the absence of data on the relationship between *chronic* consumption of L-tryptophan and the potential for adverse effects, and because of continuing uncertainty of the possible role of L-tryptophan in the development of eosinophilic fasciitis, a tolerable upper intake level (UL) was not established for L-tryptophan.

AVIS de l'Agence française de sécurité sanitaire des aliments du 16 juin 2009 relatif à l'emploi de tryptophane à hauteur de 1000 mg dans les compléments alimentaires. French Food Safety Agency (AFSSA), France, 2009

This was a brief report which addressed a request concerning the proposed upper limit for added tryptophan in food supplements. This application specifically requested an increase in the upper level of tryptophan in food supplements to 1000 mg/day (AFSSA, 2009).

AFSSA considered, based on the available literature, that side effects (fatigue, nausea and headache) were reported at tryptophan intakes of between 3 and 6 g/day. They noted that the manual for the prescription drug Optimax® sold in the UK, with the active ingredient being tryptophan and the recommended dose being 3 grams per day, listed side effects including risk of drowsiness, nausea and headache. They therefore considered the lowest observed adverse effect level (LOAEL) of tryptophan to be 3 g/day. They also noted the suggested, although not established, association between a high tryptophan consumption and risk of cataract. In addition, they cautioned against the interactions between tryptophan and a range of antidepressant and antipsychotic drugs including monoamine oxidase (MAO) inhibitors, serotonin reuptake inhibitors, benzodiazepines and phenothiazines. As the indications for these drugs corresponded with the proposed indications for use of tryptophan supplements, the Agency considered concomitant use to be highly likely.

AFSSA (2009) also reminded that the use of food supplements is not subject to medical monitoring, thus there is a risk of overdose. They therefore considered that taking into account the reported side effects, the lack of well-designed studies, and the risk of drug interactions, maintaining the uncertainty factor of 10 was justified. In conclusion, AFSSA recommended to reject the proposed threshold of 1000 mg/day and to maintain the limit of 220 mg/day for tryptophan in food supplements, as proposed by the COT in 2004 and reaffirmed in 2005. The Agency also emphasised that the quality of tryptophan must conform to European Pharmacopoeia, due to the potential risk of EMS associated with contaminants.

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

This report was an assessment of substances with nutritional or physiological effects to provide an opinion on whether their use in foodstuffs should be restricted or prohibited. The report concerned food fortification. Addition of substances to food supplements was outside the scope of the opinion. Amino acids, including tryptophan, were among a large heterogeneous group of substances reviewed (ANSES, 2011).

Although a risk assessment of L-tryptophan in food supplements was not part of this opinion, it is noteworthy that the Agency emphasised the particular limitations associated with risk assessment of amino acids, highlighting L-tryptophan by way of example, having multiple, intricate metabolic and catabolic pathways, in that very little research has been conducted on their metabolism to determine the risks of overconsumption.

Risikogruppering av aminosyrer (risk categorisation of amino acids). Opinion of the Panel on nutrition, dietetic products, novel food and allergy of the Norwegian Scientific Committee for Food Safety (VKM), Norway, 2011

In 2011, the Panel on nutrition, dietetic products, novel food and allergy in VKM published a report where they performed a qualitative risk categorisation of about 30 amino acids and amino acid compounds into three categories according to the potential risks associated with a high intake from e.g. food supplements (VKM, 2011). The substances were grouped into high, medium, or low risk. Tryptophan was one of four amino acids grouped into the high risk category, based on a broad MEDLINE search and systematic literature review. The criteria for being categorised as "high risk" included having a direct effect on organs or the central nervous system or being associated with increased risk of developing disease. The reason for classifying tryptophan as high risk was the unresolved risk of EMS.

VKM regarded this as a preliminary assessment, with the need for further risk assessments before being able to draw conclusions concerning risk of adverse health effects from

consumption of the single substances. Based on this risk grouping, a more thorough and targeted risk assessment of tryptophan was later performed by VKM (see below).

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. Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN), Spain, 2012

The Spanish Scientific Committee were asked to assess the proposal to authorise certain substances other than vitamins and minerals in the manufacture of food supplements, since vitamins and minerals were the only substances regulated at the time. The report covered assessment of 49 various substances including amino acids.

The committee proposed a daily maximum amount of L-tryptophan for use in the manufacture of food supplements of 300 mg/day. They also warned that such supplements should not be recommended for persons receiving treatment with antidepressants, nor for pregnant women or persons with renal insufficiency. The committee claimed that their recommendation was based on the protein reference intake recommended by the WHO for the adult population (WHO, 2007). It is however difficult to appraise the proposed level, as a rationale was not provided in the report. A brief overview of toxicity studies in animals was provided (see section 2.4.2), but no additional relevant studies in humans were discussed.

Risk assessment of histidine, methionine, S-adenosylmethionine and tryptophan. Opinion of the Panel on nutrition, dietetic products, novel food and allergy of the Norwegian Scientific Committee for Food Safety, Norway, 2013

This risk assessment was performed as a follow-up to the 2011 report where four amino acids were grouped as "high-risk". The opinion was based on conclusions from the IOM (2005) report and scientific publications retrieved in a systematic literature search conducted in October 2012. Five new studies in humans published after 2002 were identified in the literature search. These included one epidemiologic observational study, one experimental toxicological study with tryptophan loading, one experiment (n=1) where the investigator injected himself with quinolinic acid, a tryptophan metabolite on the kynurenine pathway, and two case reports of eosinophilia in patients using tryptophan supplements (VKM, 2013).

In the tryptophan loading study (Forrest et al., 2004) a single dose of 6 grams tryptophan was given to 15 healthy men aged 21-56 years. Blood concentrations of tryptophan and its metabolites were measured after five and seven hours. They observed 5-fold increase in tryptophan concentrations after five hours, and still significantly increased concentrations after seven hours. There was a significant increase in peroxidation products as measured by malondialdehyde after five hours by 71% and after seven hours by 109%. The concentrations of kynurenine, 3-hydroxy-antranilic acid, quinolinic acid were significantly increased after five and seven hours, while kynurenic acid, 3-hydroxykynurenine and xanthurenic acid levelled off. The authors concluded that high doses of tryptophan exhibit

oxidative action through quinolinic acid, 3-hydroxy-kynurenine and 3-hydroxy-antranilic acid, all known as for their pro-oxidative actions.

In an observational cohort study (Okada et al., 2009), 94 Japanese subjects were investigated with respect to use of tryptophan supplements and EMS development. The subjects were typed for alleles in major histocompatibility complex loci HLA-DRB1 and DQA1. Multivariate analyses were performed with tryptophan dose, age, sex and alleles and subsequent development of EMS spectrum disorder. In this study 27 subjects had EMS, 43 had EMS like syndrome, 26 had myalgia, and 18 subjects were unaffected. All subjects used tryptophan supplements, and mean dose among unaffected subjects was 2.9 g/day while mean dosage among the affected was 4.2 g/day. Higher dosage of tryptophan and age over 45 years together with HLA-DRB1*03, DRB1*04 and DQA1*0601 haplotypes, were all risk factors for development of EMS. Other HLA alleles were protective. The authors concluded that polymorphisms in the immune response genes, the xenobiotic dose and age may have implications for EMS and that this might explain why most individuals taking tryptophan supplements do not develop EMS.

In summary, VKM (2013) considered that intake of a single dose of 6 grams resulted in a significant increase in lipid peroxidation products, indicating increased oxidative stress. Intake of tryptophan supplements in doses of 4.2 g/day has been linked to the development of eosinophilia, but this question is still unresolved. Eosinophilia may have a negative health impact and hence tryptophan might still be considered to be of health concern. No dose-response studies or information concerning adverse health effects related to dose were found, thus UL could not be established. However, a tentative guidance level (GL) of 220 mg/day was suggested for tryptophan, in accordance with the level not associated with any appreciable health risk according to the UK Committee on Toxicity in 2004.

2.1.2 Literature search

Systematic searches for published literature from 2012 and up to the present (30.04.2015) were performed in MEDLINE and EMBASE in order to retrieve any recent studies on adverse effects caused by L-tryptophan published after the search included in the latest VKM report. Both databases were searched to ensure comprehensive study retrieval. The literature searches were performed by the panel coordinator in the secretariat on 30 April 2015.

In addition, a broad systematic literature search was performed in MEDLINE and EMBASE on 07 October 2015, aiming at identifying studies with tryptophan performed in children and adolescents, to, if possible, obtain information about whether tolerance may be lower in these groups. Titles and abstracts including search terms related to children or adolescents were searched for the period 1974 to present in EMBASE, and 1946 to present in MEDLINE.

The search strategies for both searches are shown in Appendix 1.

2.1.2.1 Publication selection

The study types for inclusion in this opinion have been human studies. The criteria for inclusion were:

- Tryptophan in relation to adverse effect must be addressed in the abstract
- Oral route of exposure to tryptophan in human studies
- Results not affected by other substances than tryptophan
- Human studies performed in apparently healthy individuals or patient groups who are assumed to have normal tryptophan absorption and metabolism.

In vitro studies were not included. Also papers in languages other than English, Norwegian, Danish or Swedish were excluded.

The main literature search performed 30th April 2015 identified 145 unique publications (153 hits of which 8 were duplicate hits) published in the period 2012-2015.

Study titles and abstracts were reviewed independently by two panel members according to the inclusion criteria listed above. Titles were selected if chosen by one of the experts, and resulted in retrieval of 13 full-text scientific papers. After review of the full-text papers by the author of this report, 10 papers were excluded. The reasons for exclusion were:

- Published prior to the 2013 VKM risk assessment and included in that report (1)
- Narrative reviews or subjective expert opinions without any descriptions of systematic literature search (3)
- Dose and independent effect of tryptophan cannot be established since tryptophan was provided in combination with other nutrients or as part of a tryptophan-enhanced diet by added proteins or protein hydrolysates (4)
- No mention of adverse events or harmful effects of tryptophan (1)
- Observational study with no oral administration of tryptophan (1)

The search in children and adolescents retrieved no relevant studies.

A final total of three publications were identified and included in the results in this report (see Figure 2.1.2.1-1).

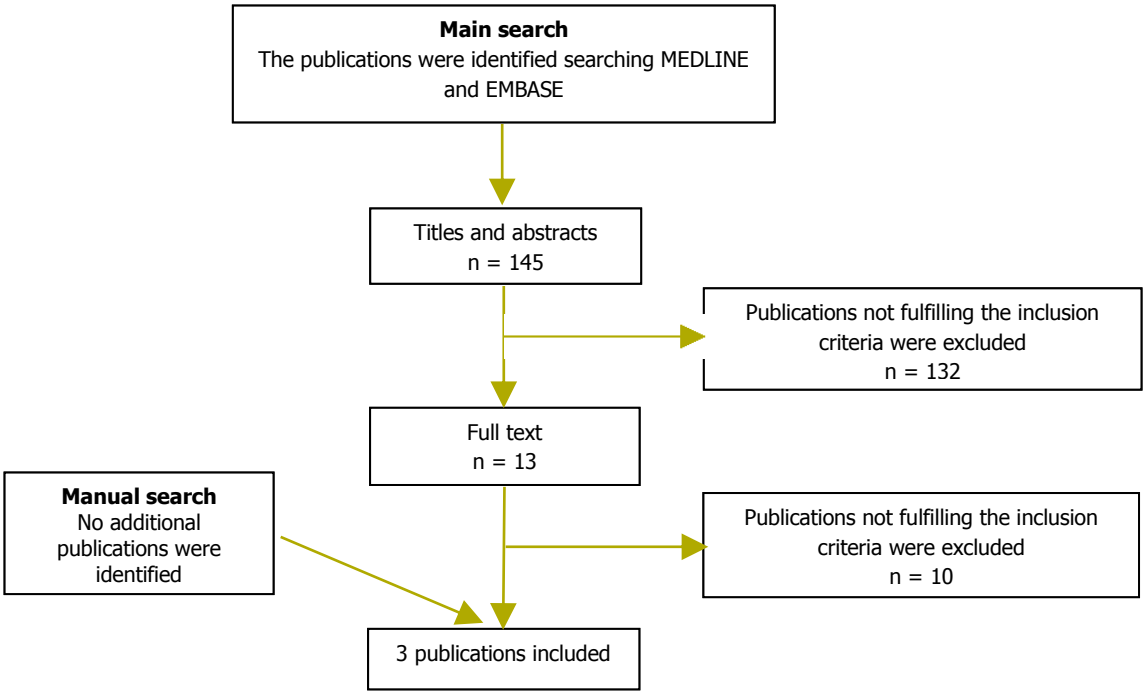

Figure 2.1.2.1-1: Flow chart for publication selection for tryptophan literature search.

2.2 General information

2.2.1 Chemistry

L-tryptophan ($C_{11}H_{12}N_2O_2$), with the chemical names *(S)-2-Amino-3-(3-indolyl)propionic acid* and *L-α-Amino-3-indolepropionic acid*, is an amino acid with CAS number 73-22-3. Figure 2.2.1-1 shows the structural formula for this amino acid. Only the L-form of amino acids can be metabolised in the human body. Absorption and metabolism of the D-form of amino acids are unknown. This assessment therefore only applies for L-tryptophan, which is the form present in proteins and in dietary supplements.

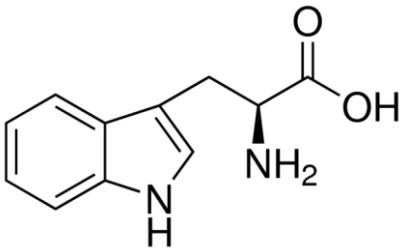

Figure 2.2.1-1: Structural formula of L-tryptophan. (Source: Sigma-Aldrich. http://www.sigmaaldrich.com/catalog/product/sial/t0254)

2.2.2 Purity

Purity is a relevant issue in relation to the concerns regarding risk of EMS after tryptophan supplementation. This was discussed in detail in the COT 2004 report (COT, 2004), see section 2.1.1.

2.2.3 Occurrence

L-tryptophan is one of the nine proteinogenic amino acids classified as indispensable (essential) in humans, as it cannot be synthesised in the human body from naturally occurring precursors at a rate sufficient to meet the metabolic requirement. Moreover, L-tryptophan is the precursor of serotonin and melatonin, as well as niacin (see section 2.3).

Tryptophan is ingested as a component of most food proteins. Examples of food sources providing tryptophan are milk and dairy products, beans, lentils, egg, meat, fish, and also bread, pasta and rice.

According to the Norwegian *Physician's Desk Reference* (*Felleskatalogen*), tryptophan is currently used as an active ingredient in combination with other amino acids in two types of prescription drugs: A peritoneal dialysis fluid indicated for patients with chronic renal failure, and an amino acid supplementation indicated for patients with chronic uremia/renal insufficiency.

2.3 Absorption, distribution, metabolism and excretion

In humans, dietary tryptophan is made available after digestion in the small intestine of the protein in which it is bound. The amino acid is absorbed as free amino acid or bound in small peptides by specific transporters in the intestinal mucosa. Tryptophan passes from the intestine to the liver where it is used as precursor for synthesis of plasma proteins and released into the blood circulation mainly as albumin. About 10-20% is in the free form in plasma (Pardridge, 1979).

Free tryptophan is the precursor for serotonin (5-hydroxytryptamine, abbreviated 5-HT), an important neurotransmitter. Serotonin is produced by the decarboxylation and hydroxylation of L-tryptophan (see Figure 2.3-1). Tryptophan ingestion raises brain tryptophan concentrations and stimulates serotonin synthesis in human brain. Increases in tryptophan may increase synthesis of the neurotransmitters also in blood and other organs (Fregly et al., 1989; Leathwood and Fernstrom, 1990; Young, 1986). Administration of 3 g L-tryptophan has been found to double the serotonin concentration in the brain (Young, 1996; Young and Gauthier, 1981). Serotonin may be further metabolised in two pathways, either into its main breakdown product 5-hydroxyindoleacetic acid (5-HIAA), or a different pathway leading to synthesis of the hormone melatonin in the pineal gland. Melatonin production affects circadian rhythm and sleep requirement. However, tryptophan competes with other large neutral amino acids (LNAA; tyrosine, phenylalanine, leucine, isoleucine and valine) for a

shared, competitive transporter across the blood-brain barrier (Fernstrom, 1983; Hawkins et al., 2006). The delivery of circulating tryptophan to the brain and its conversion to serotonin varies directly with plasma concentrations of tryptophan, while it varies inversely with the LNAA.

The rate of brain serotonin synthesis normally depends on the concentration of L-tryptophan (Fernstrom and Wurtman, 1971; Fernstrom and Wurtman, 1972). This is because tryptophan hydroxylase, the enzyme that catalyses the initial and rate-limiting step, has a very low affinity for tryptophan and is thus highly unsaturated at physiologic brain tryptophan concentrations (Lovenberg et al., 1968).

Tryptophan is also the precursor for an intermediary metabolite of a complex metabolic pathway, the so-called kynurenine pathway, ending with nicotinic acid (niacin; vitamin B_3). See Figure 2.3-1.

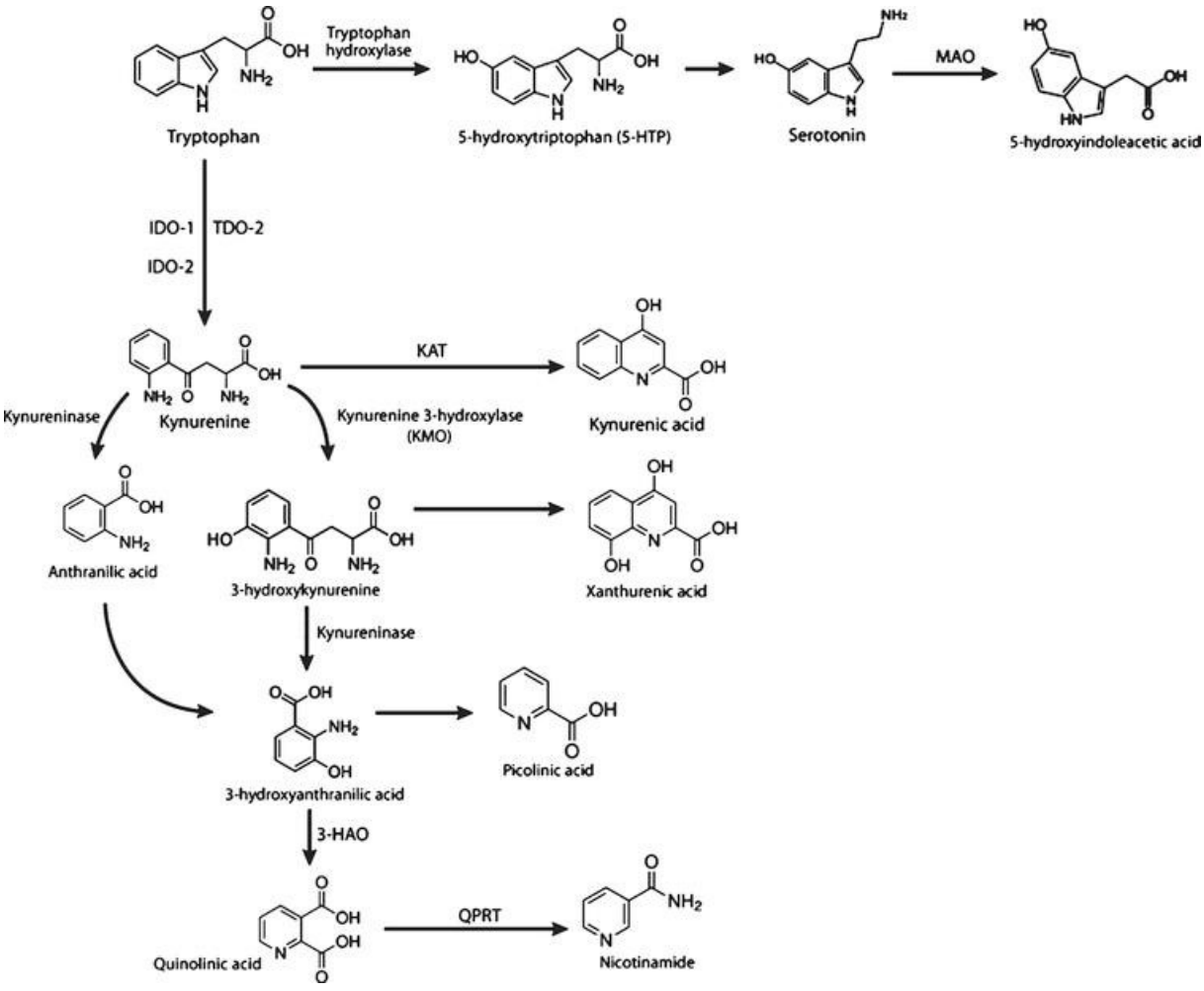

Figure 2.3-1: The serotonin and the kynurenine pathways of tryptophan metabolism (Source: Gelareh Mazareia and Blair R. Leavitta. Journal of Huntington's Disease 4 (2015) 109–118 Figure 1).

2.4 Toxicological data/Adverse effects

2.4.1 Human studies

The three relevant publications identified in the literature search included one randomised controlled trial (RCT) and results from one crossover trial reported in two separate publications. The lowest doses of L-tryptophan tested in the trials exceeded the doses specified for the current risk assessment.

Table 2.4.1-1: Randomised controlled trials investigating L-tryptophan and adverse health effects from 2012 onwards, identified in the systematic literature search

Ref.	Participant characteristics, age groups	Country	Number of participants		Doses	Main endpoints	Duration of intervention	Adverse effects
			L-tryptophan	Control				
Hiratsuka et al. (2013) and Hiratsuka et al. (2014)	Apparently healthy women aged 18-20 years	Japan	17 [1]	17 [1]	0 mg/d (placebo); 996, 1992, 2988, 3984, and 4980 mg/d	Biomarkers in blood and urine	Duration of intervention 21 days, washout period between doses 5 weeks	Monitored: blood and 24 h-urine tryptophan and its metabolites on the serotonin and kynurenine pathways. Profile of mood states. Findings: No adverse effects
Robinson et al. (2014)	Patients >=60 years undergoing major elective operations requiring a postoperative intensive care unit admission. Mean age 69 years, 98% male.	USA	152	149	3 g/d vs. placebo	Postoperative delirium	3 days	Monitored: eosinophil count, eosinophilia myalgia, serotonin syndrome. Reported subjective side effects (nausea, bad taste) Findings: No significant differences between tryptophan and placebo

[1] Randomised crossover intervention study design; all subjects received each dose level and placebo.

2.4.1.1 Randomised controlled trials (RCT)

Supplementing healthy women with up to 5.0 g/day of L-tryptophan has no adverse effects. Hiratsuka et al., 2013

This was a double-blinded randomised crossover intervention study with tryptophan supplementation to healthy young adult Japanese women (Hiratsuka et al., 2013). The objective was to determine an interim NOAEL of L-tryptophan in healthy humans ingesting up to 5 g/day tryptophan from supplements.

Twenty-one Japanese female students were enrolled, of whom 17 completed the trial (age range 18-26 years). The intervention consisted of six 21-day regimes of five different doses

of tryptophan and one placebo period, with a 5-week washout period between each intervention period.

The doses tested, were: 0 mg/day (placebo), 996 mg/day (~1 g/day), 1992 mg/day (~2 g/day), 2988 mg/day (~3 g/day), 3984 mg/day (~4 g/day), and 4980 mg/day (~5 g/day).

The intervention consisted of 20 tablets taken with one single meal, three times a day. To attain the specific daily dose, the appropriate numbers of identical-looking placebo tablets containing 83 mg gelatinised cornstarch and intervention tablets containing 83 mg tryptophan were combined. For example, the lowest tryptophan dose (996 mg) regime consisted of 4 tryptophan tablets and 16 placebo tablets given with three meals a day, while the highest tryptophan dose (4980 mg) regime consisted of 20 tryptophan tablets and 0 placebo tablets given with three meals a day.

Fasting blood samples and 24-hour urine samples were collected on the last morning of each 21-day trial period. Circulating L-tryptophan and a wide range of biomarkers on the serotonin and kynurenine pathways as well as urinary percentage of tryptophan excretion were measured. The participants also completed a standardised profile of mood states (POMS) questionnaire at the end of each trial period, yielding separate scores for the six dimensions tension-anxiety, depression-dejection, anger-hostility, vigor, fatigue, and confusion.

The whole blood levels of tryptophan, serotonin and niacin were not affected by the supplementation with up to 5 g/day tryptophan. The concentrations of metabolites including kynurenine, kynurenic acid, anthranilic acid, 3-hydroxykynurenine, xanthurenic acid and 3-hydroxyanthranilic acid were very low, trace, or undetectable even at the highest tryptophan dose. These findings indicate that blood samples were unsuitable for evaluating the dose-response effects of tryptophan loading.

Urinary excretion of tryptophan increased relative to the dose ingested, whereas the excretion of 5-hydroxyindole-3-acetic acid, a serotonin catabolite, remained relatively constant. The total and individual urinary concentrations of three niacin catabolites increased in proportion with the dose of tryptophan ingested, whereas the niacin concentration did not.

There was no effect of tryptophan dose on POMS scores.

The authors noted that based on their results, urinary 3-hydroxykynurenine appeared to be a candidate biomarker to monitor potential toxicity in response to excess tryptophan.

The authors concluded that ingestion of up to 5 g/day of L-tryptophan for 21 days had no adverse effects on healthy young Japanese women (Hiratsuka et al., 2013).

Time-dependent effects of L-tryptophan administration on urinary excretion of L-tryptophan metabolites. Hiratsuka et al., 2014

In this second publication from the crossover trial described above, giving graded doses from 0 to 5 g/day, urine metabolites were examined weekly, to investigate whether tryptophan metabolism was altered through the 21-day intervention periods. 24-hour urine profiles were determined on day –1, 7, 14 and 21 at each intervention dose (Hiratsuka et al., 2014).

It was found that urinary excretion amounts of tryptophan and some of its metabolites (kynurenic acid, 2-hydroxykynurenine, xanthurenic acid, 3-hydroxyanthranilic acid, quinolinic acid, N^1-methylnicotinamide, N^1-methyl-2-pyridone-5-carboxamide and N^1-methyl-4-pyridone-3-carboxamide) were increased at day 7, but were unchanged at days 14 and 21. Other metabolites (serotonin, 5-hydroxyindole-3-acetic acid, 2-oxoadipic acid and niacin) did not change over time, even at the highest tryptophan dose. Urinary excretion of kynurenine and anthranilic acid was low. The authors concluded that administration of tryptophan for 21 days did not substantially affect tryptophan metabolism relative to a shorter period of administration, and that tryptophan did not accumulate in healthy women.

Tryptophan supplementation and postoperative delirium — a randomized controlled trial. Robinson et al., 2014

This was a randomised, double-blind, placebo-controlled trial carried out in the Denver Veterans Affairs Medical Center, Colorado, USA, among older patients (predominantly male, mean age 69) who underwent major elective operations requiring a postoperative intensive care unit admission on the general, vascular, urological, or thoracic surgery services (Robinson et al., 2014). Patients who were admitted for emergency/urgent surgery, those on MAOI, SSRI and SNRI medications, and those with preexisting eosinophilia defined as eosinophils >6% of the blood leukocyte count were not included.

The intervention consisted of 1 g oral L-tryptophan three times per day or similarly-looking placebo. The initial dose was administered in the evening after surgery. Study drug administration was continued for nine doses (3 g/day x 3 days) or until the participant was discharged from the surgical intensive care unit.

The primary outcome of interest was incidence of the motor subtype of excitatory (mixed and hyperactive) delirium. Secondary outcomes were duration of the motor subtype of excitatory (mixed and hyperactive) delirium and incidence and duration of overall delirium.

Safety and adverse events were monitored systematically as required by the US Food and Drug Administration. All patients had their eosinophil count recorded and underwent daily evaluations for eosinophilia myalgia and serotonin syndrome. A data monitoring committee completed semiannual reviews of the study. Adverse events attributed to the study drug were recorded.

Reported side effects were unpleasant taste and nausea, and occurred in 12% of all patients combined. The proportions experiencing these side effects were slightly higher in the tryptophan group, but this was not statistically significant.

2.4.1.2 Interactions, allergic sensitisation and adjuvant effects

Concerning adverse drug interactions, the so-called *serotonin syndrome* is a well identified consequence of excessive brain serotonin levels due to concomitant intake of antidepressants (Boyer and Shannon, 2005). Symptoms may vary from mild to life-threatening. The symptoms are threefold: cognitive (headache, agitation, hypomania, mental confusion, hallucinations, coma), autonomic (shivering, sweating, hyperthermia, vasoconstriction, tachycardia, nausea, diarrhea), and somatic (myoclonus, hyperreflexia, tremor) (Dunkley et al., 2003).

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

2.4.2 Animal studies

L-tryptophan has low acute toxicity. LD_{50} in mice, rats and rabbits typically range from 2-16 g/kg bw (Herbst, 1994). The lowest reported oral LD_{50} of L-tryptophan is 1.6 g/kg bw in rats, where death was thought to have resulted from an accumulation of metabolites such as ammonia and urea (Gullino et al., 1956). Doses below the LD_{50} reduce food intake and growth of rats when given chronically. This is not specific to tryptophan, but occurs with any diet in which there is an imbalance of any amino acid (Sainio et al., 1996).

According to a review by Sainio et al. (1996), adverse events identified in experimental animals given short-term supplementation with L-tryptophan include:

- Fatty liver and ultrastructural changes in the liver of rats (Hirata et al., 1967; Trulson and Sampson, 1986)
- Enhancement of plasma lipid peroxidation in rats (Aviram et al., 1991)
- Fibrosis and acinar changes in the pancreas in rats (Love et al., 1993)
- Cytoskeletal and macromolecular permeability alterations in the small intestinal epithelium in hamsters (Madara and Carlson, 1991)
- Acute hemolytic anemia in ponies (Paradis et al., 1991)

After the 1989 EMS outbreak, numerous animal studies were performed to identify any contaminants associated with the EMS outcome (not discussed here; see section 2.1.1).

In the 2005 IOM report (IOM, 2005), the reported adverse effects of L-tryptophan supplementation in animal studies that were considered included:

- Reduced food intake and weight gain over a 4-day to 4-week period in rodents given a 5% tryptophan diet (Benevenga and Steele, 1984; Harper et al., 1970)
- Development of scaly tails and thinning hair in rats given a 20 percent casein diet supplemented with 14.3 percent tryptophan for 4 weeks (Funk, 1991)
- Decreased body weights of mice and rats when adding 2.5% or 5% L-tryptophan to their diets for 2 years (DHEW, 1978)
- Decreased weight gain in pigs supplemented with 2 or 4 percent L-tryptophan for up to 40 days, and also decreased food intake at the 4 percent-dose (Chung et al., 1991)
- Impaired maternal weight gain and reduced fetal weight in pregnant rats supplemented with 1.4 to 6 percent L-tryptophan (Funk, 1991; Matsueda and Niiyama, 1982)
- Decreased brain weights in offspring of rats when 1 percent L-tryptophan was added to diets of male and female rats beginning 2 weeks before mating, further deteriorating over three successive generations (Thoemke and Huether, 1984)

In the AESAN (2012) report, the reported adverse effects of L-tryptophan supplementation in animal studies that were considered included:

- *Acute effects*: LD_{50} between 2-16 g/kg bw in mice, rats and rabbits (Herbst, 1994)
- *Subchronic effects* by intraperitoneal administration in doses up to 2 g/kg bw per day for 30 days in rats: Hepatic damage, loss of appetite, weight loss, reduction in motor activity and eventually death (Herbst, 1994).
- *Chronic effects*: Inconsistent evidence; some studies suggest carcinogenic effects on hepatic tumors, other studies indicate no carcinogenic effects of high doses over long periods – including a long-term well-designed rat carcinogenicity bioassay conducted by the US National Cancer Institute (DHEW, 1978; Herbst, 1994). In that study, 35 rats and 35 mice were given L-tryptophan at 2.5 and 5% in drinking water, equivalent to 2.5 to 5 mg/kg bw, five times a week for 78 weeks. A tumorogenic effect or enhancer of tumour induction was not observed in any case.
- *Reproductive effects*: Reduced weight and size babies and a higher perinatal mortality rate in golden hamsters when given 1% L-tryptophan in drinking water (Herbst, 1994).

2.4.3 Mode of action for adverse effects

Common negative side effects such as drowsiness, faintness and headache are likely to be due to the action of the neurotransmitters and hormonal products of tryptophan in the nervous system. The symptoms of the *serotonin syndrome* are caused by the excessive serotonergic action on neural receptors. It has also been suggested that adverse effects may be related to the pro-oxidant effect of high L-tryptophan doses. An experimental study in 15

healthy subjects showed that tryptophan loading produced a highly significant increase in lipid peroxidation products in parallel with, and potentially resulting from, the increase in metabolic products on the kynurenine pathway (Forrest et al., 2004).

According to the AFSSA 2009 report (AFSSA, 2009), the possibly increased risk of cataract at high tryptophan levels may be caused by chemical reactions between metabolites of the kynurenine pathway, in particular xanthurenic acid and hydroxykynurenine, and the crystalline proteins in the lens, promoting their photo-oxidation and thus leading to gradual opacification developing into cataract.

Concerning potential mechanisms for EMS development, the COT (2004) report stated as follows: "There is little information on the possible mechanisms that could lead to EMS. The limited available animal data have not reproduced all the features of EMS. Administration to rats of EMS-implicated L-tryptophan and the contaminant EBT resulted in myofascial thickening. A US pharmacopoeiagrade L-tryptophan, which was not associated with cases of EMS and did not contain any EBT, also caused increased myofascial thickening but to a lesser degree. This thickening of the myofascia could be related to prior resolved inflammation rather than a direct effect of the tryptophan. The toxicological significance of this is therefore unclear."

2.4.4 Vulnerable groups

Antidepressant users: The risk related to the *serotonin syndrome*, an adverse drug reaction with potentially life-threatening symptoms occurring at excessive serotonin levels (see section 2.4.1.2), indicates that tryptophan supplements should not be ingested by patients using anti-depressant drugs. In Norway, the population incidence rate of initiating antidepressant treatment has been about 9 per 1000 inhabitants per year during the last decade. Three-fourths of these drugs are prescribed by general practitioners (Kjosavik et al., 2011). The number of prescriptions of antidepressants and other psychotropic drugs to children and adolescents has increased considerably from 2004 to 2014 (Hartz et al., 2016).

Children and adolescents: We do not have any evidence to set a tolerance level for L-tryptophan specifically for children or adolescents. Therefore, an assumption is made that these age groups have similar tolerance as adults relative to their body weight.

Pregnant and lactating women: It is not known whether moderate supplementation with tryptophan has any effect on the human fetus, or whether the tolerance is difference in pregnant and lactating women. It has been noted that the activity of tryptophan-2,3-dioxygenase, an enzyme responsible for conversion of L-tryptophan into N-formyl-L-kynurenine, may be reduced in pregnant women (AESAN (2012), citing Herbst (1994)). Caution should always be exercised for these groups. In animal studies in hamsters and rats, tryptophan supplementation has led to reduced survival of embryo and offspring, reduced offspring weight, and retarded development in the serotonin system of the offspring (Sainio et al., 1996). In the US NHANES III, the 99-percentiles of dietary L-Tryptophan intake were

higher in pregnant and lactating women than in other women (IOM, 2005), probably due to higher food intake in general.

Vulnerable groups pertaining to EMS: According to the report of COT (2004), it has not been possible to identify any particular subgroups with increased susceptibility to developing EMS, although one study showed that people with EMS were more likely to have a genotype associated with poor CYP2D6-dependent metabolism (Flockhart et al., 1994). However, the majority of EMS cases had extensive CYP2D6-dependent metabolism.

2.5 Summary of hazard identification and characterisation

Information about potential adverse effects and their associated doses has been mainly derived from the available information summarised in previous safety assessment reports, and VKM has not at the present time assessed the evidence from original publications prior to 2012. According to previous reports, short-term supplementation with L-tryptophan supplements in doses of 3 g/day and higher have led to adverse effects including appetite suppression, nausea and vomiting, faintness, dizziness, drowsiness, tremor, fatigue, and headache. A suspected, but not established, increased risk of cataract has also been reported. There is a lack of data concerning long-term supplementation.

Several doses associated with observed adverse effects have been referred to in the literature, the lowest being 3 g/day, which represents a LOAEL put forward by the AFSSA in 2009 (AFSSA, 2009).

In an older experimental study cited in the literature, 70 mg/kg bw per day has been quoted as the lowest dose associated with adverse side effects (Greenwood et al., 1974). In that study, a dose of 70 mg/kg bw per day (70 kg body weight assumed) caused an 8-fold rise in plasma tryptophan concentrations and was associated with drowsiness, clumsiness, mental slowness, and nausea. Based on the default body weight for children aged 10-<14 years (see chapter 3), this level corresponds to a dose of 3 g/day in that age group. A systematic literature search in children and adolescents with no restriction concerning publication year retrieved no relevant studies, revealing lack of data about potential adverse health effects of L-tryptophan in children and adolescents.

A crucial point is that adverse effects are exacerbated when tryptophan is administered in combination with antidepressant medications with serotonergic actions (i.e. which increase brain serotonin levels), including MAOIs, SSRIs, SNRIs tricyclic antidepressants and other drugs (Boyer and Shannon, 2005). This is a well-characterised adverse drug reaction caused by excess activation of serotonergic receptors, known as the *serotonin syndrome*.

In summary, we consider the following information in the current assessment:

- In short-term human studies, adverse effects of L-tryptophan supplementation including nausea, headache and drowsiness have been reported at doses of 3 g/day and higher, corresponding to a previously identified LOAEL (AFSSA, 2009).

- There is a lack of long-term studies in adult humans, and no relevant studies were identified in children or adolescents, while chronic studies in animals provide conflicting results with regard to carcinogeneity of L-tryptophan.
- Although data point in the direction that eosinophilia myalgia and fasciitis is likely to have been caused by impurities in food supplements, the absence of a direct causal role of L-tryptophan is not established, and the mechanisms are not understood, therefore caution should be exercised due to the severity of this syndrome.
- Adverse effects may be exacerbated when taking L-tryptophan in combination with serotonergic medications, and these interaction effects have the potential to lead to life-threatening conditions. It is notable that those obtaining prescription drugs for depression, constituting 6% of the population in Norway in 2014 (www.norpd.no), are also likely to constitute the target audience for marketing of over-the-counter tryptophan supplements.
- A NOAEL of 2228 mg/day has been identified, based on monitoring of patients treated with tryptophan prescribed as an antidepressant in the UK (COT, 2004). This was based on the average dose prescribed to more than 5000 patients, of whom 96% returned questionnaires and reported no appreciable side effects. The utility of this conclusion is limited by the missing information concerning side effects in the more than 200 patients (4%) who did not return questionnaires, and by the notion that EMS was the only adverse health effect considered in that report. It cannot be ruled out that adverse effects may occur at this dose level. Considering this uncertainty and the potential severity of adverse effects, it is prudent to apply an uncertainty factor of 10.

Based on these considerations, VKM will use 220 mg/day as a value for comparison in the risk characterisation of L-tryptophan, corresponding to 3.1 mg/kg bw per day in a 70 kg adult. This value corresponds to the upper safe supplemental tryptophan dose proposed by the COT in 2004 (COT, 2004), supported by the AFFSA in their conclusion in 2009 (AFSSA, 2009), and again put forward as a tentative guidance level by VKM in 2013 (VKM, 2013).

3 Exposure

3.1 Food supplements

The Norwegian Food Safety Authority has requested a risk assessment of the doses 250 mg/day, 300 mg/day, and 450 mg/day of L-tryptophan in food supplements for children 10 years and older, adolescents and adults. We do not have any evidence to set a tolerance level for L-tryptophan specifically for children or adolescents. Therefore, an assumption is made that these age groups have similar tolerance as adults relative to their body weight.

The default body weights (bw) for these groups determined by the EFSA were used: 10-<14 years=43.4 kg, 14-<18 years=61.3 kg, and adults=70 kg. The resulting intakes per kg bw according to each of the three supplement doses is given in Table 3.1-1.

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

Age group	Daily dose, mg	Body weight (kg)	Exposure (mg/kg bw per day)
Children (10-<14 years)	250 300 450	43.4	5.8 6.9 10.4
Adolescents (14-<18 years)	250 300 450	61.3	4.1 4.9 7.3
Adults (≥18 years)	250 300 450	70.0	3.6 4.3 6.4

3.2 Other sources

There is no data available concerning dietary intake of L-tryptophan in Norway. In the USA, estimated mean usual daily intake of tryptophan from food and supplements was found to be 910 mg/day and 1790 mg/day in the 99th percentile, based on data from the third National Health and Nutrition Examination Survey (NHANES III). Men aged 51-70 had the highest intake, with the 99th percentile of 2110 mg/day (IOM, 2005).

This corresponds to the older UK National Food Survey of 1974 which indicated an average dietary intake of L-tryptophan of 890 mg/day (Buss and Ruck, 1977) cited in COT (2004).

4 Risk characterisation

The doses received from NFSA are 250, 300 and 450 mg/day L-tryptophan in food supplements, and the exposures for adults, adolescents and children above 10 years are given in chapter 3.

The value for comparison used in this risk characterisation is 220 mg/day, corresponding to 3.1 mg/kg bw per day in a 70 kg adult.

There are no studies in children or adolescents. Nor are there any data indicating that children and adolescent are less vulnerable than adults for L-tryptophan. No tolerance level is set for L-tryptophan specifically for children or adolescents, thus assuming similar tolerance for these age groups as for adults.

The estimated exposure per kg body weight at the predefined supplement doses exceeds the value for comparison in all age groups considered in this report. Thus, VKM considers that:

- In adults (≥18 years), the specified doses 250, 300, and 450 mg/day L-tryptophan in food supplements may represent a risk of adverse health effects.
- In adolescents (14 to <18 years), the specified doses 250, 300, and 450 mg/day L-tryptophan in food supplements may represent a risk of adverse health effects.
- In children (10 to <14 years), the specified doses 250, 300, and 450 mg/day L-tryptophan in food supplements may represent a risk of adverse health effects.

5 Uncertainties

There is scarce evidence concerning tryptophan doses consistent with potential adverse effects in the population (see chapter 6). Although several reports have addressed the topic, they have all communicated a large degree of uncertainty. We have applied an uncertainty factor of 10, representing the default uncertainty factor to allow for within-species variability in humans. We consider an uncertainty factor of 10 to be justified in light of the potential mechanisms and the severity of adverse effects previously reported at high doses.

There is also a risk that our search strategy has failed to identify studies that have reported adverse effects as not all RCTs report adverse effects in a similar manner and according to existing guidelines such as those suggested by the "Consort group" (http://www.consort-statement.org).

6 Data gaps

- Lack of data in children and adolescents: A systematic literature search in children and adolescents with no restriction concerning publication year retrieved no relevant studies, revealing lack of data about potential adverse health effects of L-tryptophan in children and adolescents.
- Lack of human toxicity studies on adverse effects as primary outcome of tryptophan supplementation, with the possibility to establish a dose-response relationship: The majority of intervention studies are designed to detect health-promoting effects of tryptophan. Also, many of them are performed in selected patient groups including e.g. severe depression and psychosis, ADHD, or conditions involving metabolic disturbances. As is not uncommon in supplementation studies, tryptophan was in several studies, excluded from this assessment, provided in combination with other nutrients or as part of a tryptophan-enhanced diet by added proteins or protein hydrolysates. There is a lack of human experimental studies that are
    - Well-designed (randomised, blinded, placebo-controlled, multicenter)
    - With pure tryptophan given as a single supplement as the intervention
    - With graded doses
    - Of sufficient sample size
    - Designed to study long-term effects – i.e. sufficient duration of intervention and sufficient duration of follow-up
    - Performed in apparently healthy subjects representative of the general population

7 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-tryptophan in food supplements at the following doses: 250 mg/day, 300 mg/day and 450 mg/day for the general population, ages 10 years and above.

According to previous reports, doses above 3 g/day of L-tryptophan from supplements have been associated with adverse effects. In addition, purity is an important issue, since observed adverse effects of supplementation have resulted from impurities in the manufacture of tryptophan supplements. Conclusions in previous reports have suggested maximum levels of 300 and 220 mg/day, respectively. An upper level of 220 mg/day was first proposed by the UK Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) in 2004, and was based on the average dose of L-tryptophan consumed as a prescription drug in the UK at the time. This level has been maintained in later reports by other committees, most recently VKM in 2013. Additional publications retrieved in the literature search were not considered to be of sufficient impact to change the conclusions based on the existing knowledge base. No evidence was found to assume a specific tolerance level for L-tryptophan for children or adolescents. Therefore, a similar tolerance as for adults relative to body weight was assumed for these age groups.

The value for comparison used was 220 mg/day, corresponding to a dose of 3.1 mg/kg bw per day in a 70 kg adult, derived from the UL proposed by the UK COT (2004) and later supported by the French AFSSA (2009) and the Norwegian VKM report (VKM, 2013). For all doses considered, the estimated exposure per kg body weight for adults, adolescents and children at or above 10 years exceeded the value for comparison.

VKM concludes that:

- In adults (≥18 years), the specified doses 250, 300, and 450 mg/day L-tryptophan in food supplements may represent a risk of adverse health effects.
- In adolescents (14 to <18 years), the specified doses 250, 300, and 450 mg/day L-tryptophan in food supplements may represent a risk of adverse health effects.
- In children (10 to <14 years), the specified doses 250, 300, and 450 mg/day L-tryptophan in food supplements may represent a risk of adverse health effects.

An overview of the conclusions is presented in Table 7-1.

Table 7-1: An overview of the conclusions for L-tryptophan in food supplements.
Red: Estimated exposures to L-tryptophan may represent a risk of adverse health effects.

	L-tryptophan		
Doses Age groups	250 mg/day	300 mg/day	450 mg/day
Children (10 to <14 years)			
Adolescents (14 to <18 years)			
Adults (≥18 years)			

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9 Appendix 1

Search strategies for this risk assessment

Search strategy for human studies

Databases: Ovid MEDLINE(R) <1946 to April Week 4 2015>; Embase <1974 to 2015 April 29>. Search date: 30 April 2015

1. tryptophan*.ti. (30294)
2. (risk* or safety or adverse or side-effect*1 or hazard* or harm* or eosinophil* or negative or contraindicat* or contra-indicat* or interact* or toxicity or toxic).tw. (8772374)
3. 1 and 2 (5146)
4. (conference abstract* or letter* or editorial*).pt. (4382040)
5. 3 not 4 (4861)
6. limit 5 to (danish or english or norwegian or swedish) (4622)
7. limit 6 to human (1625)
8. remove duplicates from 7 (970)
9. limit 8 to yr="2012 -Current" (153)

Search strategy for studies in children and adolescents

Database: Embase <1974 to 2015 October 06>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily, Ovid MEDLINE(R) and Ovid OLDMEDLINE(R) <1946 to Present>. Search Date: 07.10.2015

1. tryptophan*.ti. (31640)
2. (child* or adolescent* or teenage* or college* or high school).tw. (2923769)
3. 1 and 2 (466)
4. limit 3 to (danish or english or norwegian or swedish) (352)
5. remove duplicates from 4 (219)