Risk assessment of "other substances" - L-proline

Opinion of the Panel on Nutrition, Dietetic Products, Novel Food and Allergy of the Norwegian Scientific Committee for Food Safety
Risk assessment of "other substances" - L-proline

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Assessed and approved

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Acknowledgment

The Panel on Nutrition, Dietetic Products, Novel Food and Allergy has answered the request from the Norwegian Food Safety Authority. Project leader from the VKM secretariat has been Bente Mangschou. Kristin Holvik is acknowledged for her valuable work on this opinion. Jan Alexander (the Scientific Steering Committee), Åshild Kroghdahl (the Scientific Steering Committee) and Helle Margrete Meltzer (former member of the Panel on Nutrition, Dietetic Products, Novel Food and Allergy) constituted a reference group and are acknowledged for their valuable comments and suggestions on this opinion.

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" 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-proline in food supplements, and it is based on previous risk assessments and articles retrieved from literature searches.

According to information from NFSA, L-proline is an ingredient in food supplements sold in Norway. NFSA has requested a risk assessment of 50, 500, 1000, 1500 and 1800 mg/day of L-proline from food supplements.

L-proline is considered a non-essential amino acid as it can be synthesised from arginine via the urea cycle in liver, and from glutamine/glutamic acid in the intestinal epithelium. In addition, L-proline is ingested through the diet. All protein rich foods provide L-proline, and animal proteins from milk and meat are particularly abundant sources. A dietary requirement for proline in healthy humans has not been estimated since proline is not considered an essential amino acid. Data on dietary intake of L-proline in Norway are not available. In the third US National Health and Nutrition Examination Survey (NHANES III; 1988-1994), overall mean intake of L-proline from food and supplements was 5.2 g/day.

A previous report from the Institute of Medicine (2005) cited one small uncontrolled patient study (n=4) and two animal studies, none of which assessed the toxicity of L-proline in a dose-response manner. The report concluded that a tolerable upper intake level for L-proline could not be determined.

In a risk grouping of amino acids from VKM (2011), proline was categorised as having potentially moderate risk, based on the scarce literature and the notion that amino acids are generally bioactive compounds. It was stated that "no conclusion can be drawn on a scientific basis due to lack of adequate scientific literature. Nor will it be possible to conduct a risk assessment until further studies are available".

Three systematic literature searches without restriction on publication year were performed for the current risk assessment, aimed at identifying adverse effects of L-proline.
supplementation in human and animal studies. In humans, one uncontrolled experimental study was identified where a single oral dose of 500 mg/kg bw L-proline was administered as a growth hormone stimulatory agent to 20 children with hyposomatotropic dwarfism and 20 healthy children. No adverse effects were observed. In animals, one relevant subchronic (90 days) toxicological dose-response study in rats was included and forms the basis for the current risk assessment. In that study, performed in accordance with official guidelines from the Japanese Ministry of Health, Labour and Welfare, there were no indications of toxicity at the highest dose given through a powder diet (5.0% L-proline). This dose corresponded to 2773 mg L-proline/kg bw per day and was used as a no-observed-adverse-effect-level (NOAEL).

Studies to set a tolerance level for L-proline for children or adolescents have not been found. Therefore, an assumption is made that these age groups have similar tolerance as adults relative to their body weight.

To evaluate the safety of the specific doses given by NFSA, margin of exposure (MOE) was calculated with use of 2773 mg L-proline/kg bw per day as NOAEL. For the highest dose (1800 mg/day) MOE is 67 (= 2773* 43.3/1800) in children 10 to <14 years (default body weight 43.3 kg), and 94 (= 2773* 61.3/1800) in adolescents 14 to <18 years (default body weight 61.3 kg). For the dose of 1500 mg/day, the MOE in children is 80. MOE for all the other doses and age categories are above 100.

Based on the magnitude of MOE, the lack of adverse effects at the highest dose tested (current NOAEL) and the notion that L-proline is a nutrient that is synthesised endogenously from other amino acids in addition to a dietary intake in the magnitude of 5 grams per day, VKM concludes that:

- In adults (≥18 years), the specified doses 50, 500, 1000, 1500 and 1800 mg/day L-proline in food supplements are unlikely to cause adverse health effects.
- In adolescents (14 to <18 years), the specified doses 50, 500, 1000, 1500 and 1800 mg/day L-proline in food supplements are unlikely to cause adverse health effects.
- In children (10 to <14 years), the specified doses 50, 500, 1000, 1500 and 1800 mg/day L-proline 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-proline in food supplements. VKM concludes that:

- In adults (≥18 years), the specified doses 50, 500, 1000, 1500 and 1800 mg/day L-proline in food supplements are unlikely to cause adverse health effects.
• In adolescents (14 to <18 years), the specified doses 50, 500, 1000, 1500 and 1800 mg/day L-proline in food supplements are unlikely to cause adverse health effects.
• In children (10 to <14 years), the specified doses 50, 500, 1000, 1500 and 1800 mg/day L-proline in food supplements are unlikely to cause adverse health effects.

**Key words:** L-proline, 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.


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

Ifølge informasjon fra Mattilsynet er L-prolin en ingrediens i kosttilskudd som selges i Norge. Oppdraget fra Mattilsynet var å risikovurdere følgende doser av L-prolin i kosttilskudd: 50, 500, 1000, 1500 og 1800 mg/dag.


En rapport fra Institute of Medicine i USA publisert i 2005 siterte en liten ukontrollert pasientstudie (n=4) og to dyrestudier, hvorav ingen vurderte dose-respons-toksisitet av L-prolin. Rapporten konkluderte med at det ikke kunne fastsettes et øvre tolerabelt inntaksnivå for L-prolin.

I en risikogruppering av aminosyrer publisert av VKM i 2011 ble prolin kategorisert som en aminosyre med potensielt moderat risiko, ettersom det finnes lite dokumentasjon for prolin og aminosyrer generelt er bioaktive komponenter. Det ble understreket i rapporten at det ikke kan trekkes noen konklusjon for L-prolin på vitenskapelig grunnlag på grunn av mangel på dokumentasjon, og at det heller ikke vil være mulig å gjøre en risikovurdering av L-prolin før man har flere tilgjengelige studier.

Tre systematiske litteratursøk uten avgrensning på publikasjonsår ble gjort for denne risikovurderingen. Litteratursøkene tok sikte på å identifisere negative helseeffekter av tilskudd med L-prolin i henholdsvis humanstudier og dyrestudier. Det ble funnet én humanstudie hvor en enkeldose L-prolin (500 mg per kg kroppsvekt) ble gitt som
stimuleringsmiddel for veksthormon til 20 barn med hypofysær vekshemmning og 20 friske barn. Ingen negative helseeffekter ble observert.

Fra litteratursøket på dyr ble én relevant subkronisk (90 dager) toksikologisk dose-responsstudie i rotter inkludert som danner grunnlaget for denne risikovurderingen. Studien ble gjennomført i samsvar med offisielle retningslinjer fra det japanske helsedepartementet. Det var ingen tegn på toksisitet ved den høyeste do森 som ble gitt i studien, 5,0 % L-prolin via pulverdiet. Denne dosen, som tilsvarer 2773 mg L-prolin per kg kroppsvekt per dag, ble satt som NOAEL (“no observed adverse effect level”).

Det er ikke funnet noen holdepunkter for et annet toleransenivå for L-prolin hos barn og ungdom enn hos voksne. En antakelse om samme toleranse hos barn og ungdom som voksne per kg kroppsvekt legges derfor til grunn i denne riskovurderingen.

For å vurdere dosene fra Mattilsynet ble "margin of exposure" (MOE) beregnet ved å benytte 2773 mg L-prolin per kg kroppsvekt per dag som NOAEL. For den høyeste dosen (1800 mg/day) utgjør MOE en faktor på 67 (=2773*43,3/1800) for barn (10 til <14 år; standard kroppsvekt 43,4 kg) og en faktor på 94 (=2773*61,3/1800) for ungdom (14 til <18 år; standard kroppsvekt 61,3 kg). MOE for dosen 1500 mg/dag utgjør en faktor på 80 hos barn.

Tatt i betraktning størrelsesorden på sikkerhetsmarginene, fraværet av negative helseeffekter ved den høyeste utprøvde dosen (NOAEL) og at L-prolin er et næringsstoff som dannes endogent fra andre aminosyrer og dessuten inntas fra kosten i størrelsesorden 5 gram per dag, konkluderer VKM at:

- For voksne (≥18 år) er det usannsynlig at de spesifiserte dosene på 50, 500, 1000, 1500 og 1800 mg/dag L-prolin i kosttilskudd vil forårsake negative helseeffekter.
- For ungdom (14 til <18 år) er det usannsynlig at de spesifiserte dosene på 50, 500, 1000, 1500 og 1800 mg/dag L-prolin i kosttilskudd vil forårsake negative helseeffekter.
- For barn (10 til <14 år) er det usannsynlig at de spesifiserte dosene på 50, 500, 1000, 1500 og 1800 mg/dag L-prolin 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-prolin i kosttilskudd. VKM konkluderer med at:

- For voksne (≥18 år) er det usannsynlig at de spesifiserte dosene på 50, 500, 1000, 1500 og 1800 mg/dag L-prolin i kosttilskudd vil forårsake negative helseeffekter.
• For ungdom (14 til <18 år) er det usannsynlig at de spesifiserte dosene på 50, 500, 1000, 1500 og 1800 mg/dag L-prolin i kosttilskudd vil forårsake negative helseeffekter.
• For barn (10 til <14 år) er det usannsynlig at de spesifiserte dosene på 50, 500, 1000, 1500 og 1800 mg/dag L-prolin i kosttilskudd vil forårsake negative helseeffekter.
Abbreviations and glossary

Abbreviations

AFSSA - Agence Francaise de Securite Sanitaire des Aliments (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
EFSA - European Food Safety Authority
GOT - glutamic-oxalic-transaminase
GPT - glutamic-pyruvic-transaminase
GSA - glutamate γ-semialdehyde
IOM - Institute of Medicine
LAT1 - L-type amino acid transporter 1
LAT2 - L-type amino acid transporter 2
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
OAT - ornithine aminotransferase
P5C - pyrroline 5-carboxylate
P5CDH - pyrroline 5-carboxylate dehydrogenase
PYCR - pyrroline-5-carboxylate reductase
P5CS - pyrroline 5-carboxylate synthase
POX - proline oxidase (same as PRODH)
PRODH - proline dehydrogenase (same as POX)
RCT - randomised controlled trial
ROS - reactive oxygen species
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 Health 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.
"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-proline in food supplements at the following doses: 50, 500, 1000, 1500 and 1800 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-proline 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-proline is an ingredient in food supplements sold in Norway. NFSA has requested a risk assessment of the intake of 50, 500, 1000, 1500 and 1800 mg/day L-proline per day from food supplements. The total L-proline exposure from other sources than food supplements is not included in the risk assessment.

L-proline is one of the 20 amino acids used by the human body. It is classified as a non-polar amino acid with an aliphatic side chain. It is considered non-essential (or dispensable) as it can be synthesised in the body from arginine and glutamine/glutamic acid, provided a sufficient supply of total protein. However it is also sometimes referred to as semi-essential or conditionally indispensable, meaning that under certain circumstances (growth and wound repair) endogenous synthesis may not be able to meet metabolic needs and dietary supply may be required. L-proline is a constituent of proteins and plays an architectural role in protein folding due to its structure. A systematic bioinformatics study found that proline residues constitute on average 6% of the amino acids in human proteins, ranging from 0 to 40% (Morgan and Rubenstein, 2013). Proline is particularly abundant in collagens.

All protein rich foods provide L-proline. Animal proteins from milk and meat are particularly good proline sources (USDA Food Composition Database, https://ndb.nal.usda.gov/). A dietary requirement for proline in healthy humans has not been estimated since proline is not considered an essential amino acid. According to the third National Health and Nutrition Examination Survey (NHANES III; 1988-1994), the overall mean intake of L-proline from food and food supplements in the United States was 5.2 g/day (IOM, 2005).
2 Hazard identification and characterisation

2.1 Literature

This risk assessment is based on previous risk assessments of L-proline, as well as scientific papers retrieved from a systematic literature search. In total, three separate literature searches were performed: One search in literature published before 6 April 2016 aiming at retrieving human studies on adverse effects caused by L-proline, one search in literature published before 3 August 2016 aiming at retrieving animal model studies on toxicity of L-proline, and finally a search in literature published before 26 October 2016 tailored to identify any studies on L-proline in children or adolescents.

2.1.1 Previous risk assessments

The safety of proline has been discussed briefly in previous reports by the Institute of Medicine (IOM) in USA in 2005, the French Food Agency (AFSSA) in 2007, the European Food Safety Authority (EFSA) in 2008, the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) in 2011, and the Norwegian Scientific Committee for Food Safety (VKM) in 2011.

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

According to the IOM (2005), there were minimal data on adverse effects of L-proline in either experimental animals or humans. Concerning animal data, two studies were cited. A study published in a textbook about amino acids in 1990 (Kampel et al., 1990) reported the results of a 30-day feeding study of female Sprague-Dawley rats. The rats were divided into groups and fed L-proline or other amino acids in drinking water; mean 50 mg/kg bw per day. A control group was also included. No histological changes were seen in the rats fed L-proline. Serum parameters for glutamic-oxalic-transaminase (GOT), glutamic-pyruvic-transaminase (GPT), alkaline phosphatase, gamma glutamyl transferase, lactate dehydrogenase, hydroxybutyric dehydrogenase and creatinine were normal in the L-lysine rats and the control rats. The NOAEL in this study was therefore 50 mg/kg bw per day.

The second animal study cited by the IOM was a male mouse model for hyperprolinemia using genetically hyperprolinemic mice (Baxter et al., 1985). These mice have 6 to 7 times the brain proline concentrations and 10 times the plasma proline concentrations compared with control animals. The study investigated learning abilities, i.e. footshock avoidance acquisition in a T-maze and a shuttlebox, respectively, comparing the performance of the
hyperprolinemic mice with that of control mice with normal brain proline levels (CD-1 mice). The genetically hyperprolinemic mice had a significant deficit for T-maze learning, but a significantly greater aptitude for shuttlebox learning when compared to the CD-1 mice. These differential learning abilities did not appear to be related to different brain levels of proline. According to the authors, the results were thus not in support of the hypothesis that high proline levels in the brain and blood would be associated with impaired memory and learning abilities.

Concerning human data, one study was cited (Hayasaka et al., 1985). This was a report from patient observations in Japan where supplementation with proline (isomer not stated), with or without vitamin B₆, had been tried to four patients with gyrate atrophy of the choroid and retina. The aim was to slow the progression of the atrophy. The patients were between 4 and 32 years old at start of supplementation. Two of the patients received proline only while two patients received a combination of proline and vitamin B₆. The proline doses ranged from 2 to 10 g/day and the treatment lasted for up to 5 years. The IOM report summarises that "no overt adverse effects were reported; however, it was uncertain from the paper which effects were studied".

The IOM concluded that no data were available for dose-response assessment and a tolerable upper intake level (UL) could not be established.

**Protein intake: Dietary intake, quality, requirements and recommendations. Agence Francaise de Securité Sanitaire des Aliments (AFSSA), France, 2007**

This French report discussed requirements and recommendations for proteins and amino acids. The report cited the rat study by Kampel et al. which did not observe any harmful changes in Sprague-Dawley rats fed 50 mg/kg bw per day of L-proline in drinking water for one month (Kampel et al., 1990). A risk assessment of proline was not performed.


This report evaluated amino acids as flavouring substance. However no risk assessment of L-proline was performed. Rather, the Panel concluded with "no safety concern" because "human exposure through food is orders of magnitude higher than the anticipated level of exposure from use as a flavouring substance".
Opinion of the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) on the assessment of the risks associated with substances with nutritional or physiological effects with a view to restricting or prohibiting their use in foodstuffs. France, 2011

The Agency did not perform a risk assessment of amino acids, and the safety of L-proline was not evaluated. Rather, the Agency made a general consideration that "for vitamins and minerals and "other substances" for which there are no reference values, ANSES considers that only the demonstration of a specific nutritional/physiological benefit covered by generic or specific claims authorised by the European Commission, following an opinion issued by EFSA, should allow their addition to food, subject to their safety of use being demonstrated."


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). The amino acids were categorised into high, moderate or low risk. The report can only be regarded as an initial screening and not as a risk assessment. For L-proline, one study was cited in the report. This was a small clinical study testing a single dose (500 mg/kg bw) L-proline as a stimulating agent for growth hormone release in nine sexually immature children aged 5-9 years hospitalised for alleged hypopituitarism (Popa et al., 1977). The results showed that serum growth hormone rose adequately (>5 ng/ml) within three hours following L-proline administration. Considering the notions that there is scarce evidence and that amino acids are generally bioactive compounds, the Panel categorised proline as having potentially moderate risk. It was also noted that "no conclusion can be drawn on a scientific basis due to lack of adequate scientific literature. Nor will it be possible to conduct a risk assessment until further studies are available".

2.1.2 Literature search

Systematic literature searches aiming at retrieving publications on adverse effects caused by L-proline were performed in MEDLINE and EMBASE with no restriction on publication year. Both databases were searched to ensure comprehensive study retrieval. The search for human studies was conducted 6 April, 2016, and the search for animal studies was conducted 3 August, 2016. The additional search for studies in children or adolescents was conducted 26 October, 2016. All three searches were limited to publications in English or Scandinavian languages. Conference abstracts, Editorials and Letters were not included in the searches. The search strategies are outlined in Appendix 1.
2.1.2.1 Publication selection and data extraction

The literature searches identified 1179 titles and abstracts; 586 in the search for human studies, 542 in the search for animal studies, and 51 in the search for studies performed in children or adolescents. All titles and abstracts were screened against the following inclusion criteria:

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

In vitro studies were not included.

The inclusion criteria checklist was developed by members of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics and the Panel on Nutrition, Dietetic Products, Novel Food and Allergy. Titles and abstracts that did not fulfil the inclusion criteria were excluded. Titles and abstracts of unresolved relevance to the current risk assessment were retained for further review. The primary screening was performed independently by two Panel members. The publications that passed the primary screening were reviewed in full text against the same inclusion criteria by the author of this report.

The first screening of titles and abstracts of human studies identified no relevant publications. The first screening of titles and abstracts of animal studies resulted in inclusion of 4 publications, of which 2 publications were excluded after full-text review. After the screening of titles and abstracts in the search for studies in children and adolescents, one paper was identified and retained after full-text review. Thus, 2 animal studies and one study performed in children were found relevant and included in the results in this report (see Figure 2.1.2.1-1).
2.2 General information

2.2.1 Chemistry

L-proline, also known as L-pyrrolidine-2-carboxylic acid, is a neutral amino acid. It is classified as non-essential, as the daily requirement is covered by endogenous synthesis from arginine and glutamine/glutamic acid, provided a sufficient supply of total protein. However it is also sometimes referred to as conditionally essential (IOM, 2005), implying that dietary supply of proline is required in certain situations when endogenous synthesis cannot meet metabolic needs due to accelerated protein catabolism in response to starvation, infection, or severe trauma such as injuries and burns (Jaksic et al., 1991). Although proline is classified as an amino acid, it is strictly speaking an imino acid, since it contains an imino
group (carbon-nitrogen double bond). Due to its cyclic pyrrolidine side chain it is classified as a nonpolar aliphatic amino acid. The molecular formula is $\text{C}_3\text{H}_9\text{NO}_2$. The CAS number for L-proline is 147-85-3. The structural formula is shown in Figure 2.2.1-1.

![Structural formula of L-proline](image)

**Figure 2.2.1-1:** Structural formula of L-proline.

### 2.2.2 Occurrence

**In the human body:** The proline content of proteins is highly variable, and it is particularly abundant in collagens. A systematic bioinformatics study found that proline residues constituted on average 6.3% of the amino acids in human proteins, ranging from 0 to 40% between the proteins in the human proteome (Morgan and Rubenstein, 2013). The proline content in a 70 kg male with moderate lipid stores and body protein level of about 15% (10.5 kg protein) may thus be approximated to 600-700 g.

**In food:** All protein rich foods provide L-proline. Animal proteins from milk and meat are particularly good proline sources (USDA Food Composition Database, [https://ndb.nal.usda.gov/](https://ndb.nal.usda.gov/)). Data on dietary intake of L-proline in Norway is not available. In the NHANES III (1988-1994) in the United States, overall mean intake of L-proline from food and supplements was 5.2 g/day. L-proline is also available in food supplements.

### 2.3 Absorption, distribution, metabolism and excretion

**Absorption:** Proline-containing dipeptides and tripeptides are efficiently absorbed throughout the small intestine by the hydrogen ion/peptide cotransporters (Kohlmeier, 2015). Free proline and hydroxyproline enter the small intestinal cells via the sodium-dependent transporter IMINO (Urdaneta et al., 1998 cited in Kohlmeier, 2015), which may also be chloride-dependent. The rBAT (SLC3A1)-anchored amino acid transporter BAT1/b0,+ (SLC7A9) facilitates entry by exchange with another neutral amino acid (Chairoungdua et al., 1999 cited in Kohlmeier, 2015), which can occur in either direction depending on the concentration gradient.

**Transport and uptake in cells:** L-proline is transported into the blood cells by cell specific mechanisms. Free circulating L-proline is taken up by tissues usually by system L, including the specifically identified heteroexchanger LAT1 (Kohlmeier, 2015). L-proline is transferred from mother to fetus mediated by LAT1 and LAT2 (Ritchie & Taylor 2001, Kudo & Boyd 2001)
cited in Kohlmeier, 2015), of which the driving force is the concentration gradient of small neutral amino acids (glycine, alanine, cysteine) established by the sodium-dependent transport systems A and ASC. L-proline also crosses the blood-brain barrier (neuroendothelial cell layer) mediated by system L, and is taken up by neurons in the brain mediated by the sodium chloride-dependent proline co-transporter (PROT) (Kohlmeier, 2015).

Endogenous synthesis: Proline can be synthesised in the body from the amino acids arginine and glutamine/glutamate (Figure 2.3-1) (Herzfeld et al., 1977; Kohlmeier, 2015; Liu and Phang, 2013; Watford, 2008). The major proportion of proline synthesis in the body occurs via the glutamate/ pyrroline 5-carboxylic acid (P5C) synthase pathway in the gut epithelium (Watford, 2008), where it is released in the portal circulation together with amino acids absorbed from food.

The relative contributions of arginine and glutamine/glutamate to endogenous proline synthesis are not known, but are assumed to depend on nutritional status, and are also tissue dependent. In tissues with a particularly high demand for proline, such as cartilage and bone, there is a high abundance of enzymes on the ornithine aminotransferase (OAT) pathway and a low presence of enzymes on the P5C biosynthetic pathway, thus favouring proline synthesis, while proline oxidation is relatively larger in other tissues (Smith & Phang 1978 cited in AFSSA, 2007). According to a rat study, about 7% of glutamine carbon metabolised in the small intestine was utilised in proline synthesis (Windmueller & Spaeth 1974, cited in Watford, 2008). VKM has not identified any information which would enable quantification of the magnitude of daily endogenous synthesis of L-proline.

Metabolism: Proline metabolism is closely related with glutamine metabolism, TCA cycle, urea cycle and pentose phosphate pathway (Figure 2.3-1). Proline metabolism is distinct from that of primary amino acids. The inclusion of an alpha-nitrogen within its pyrrolidine ring precludes it from being the substrate for the usual amino acid-metabolising enzymes, such as decarboxylases, aminotransferases, and racemases. Instead, proline metabolism has its own family of enzymes with their tissue and subcellular localisation and their own regulatory mechanisms (Liu and Phang, 2013).
Proline and hydroxyproline constitute about one-third of collagen, which is the most abundant protein in the body and has an important structural role. Collagen is produced by fibroblasts and osteoblasts and is particularly abundant in connective tissues such as in skin, vasculature, cartilage and bone (van der Rest and Garrone, 1991). Proline is hydroxylated to form hydroxyproline after its incorporation into procollagen. Hydroxyproline is an important constituent of the cross-linking of collagen chains and responsible for stabilising the triple-helix structure of collagen.

Breakdown of proline in the small intestine is the main source of citrulline, ornithine and arginine in the body (Kohlmeier, 2015). Catabolism of L-proline starts with its oxidation mediated by proline dehydrogenase (PRODH), also called proline oxidase (POX), forming P5C. Release of reactive oxygen species (ROS) occurs in the oxidation of proline (Donald et al., 2001). Proline has thus been proposed to be a stress substrate in the microenvironment of inflammation and tumorigenesis, playing a role in inducing apoptosis through intrinsic and extrinsic pathways (Phang et al., 2008).
2.4 Toxicological data/ Adverse effects

2.4.1 Human studies

The assessment of GH reserve in normal and pituitary dwarfed children by L-proline loading in high dosage (500 mg/kg). Popa et al., Rev Roum Med Endocrinol 1982

In this clinical experimental study, 20 children with hyposomatropic dwarfism and 20 healthy children of both sexes were given a single oral dose corresponding to 500 mg/kg bw (actual dose unique to each individual) L-proline dissolved in 150 ml tap water as a stimulatory agent for growth hormone (Popa et al., 1982). Due to the nature and purpose of the study it was not randomised and did not include a placebo control. The patients were aged 6-19 years (mean age 12 years), while the controls were aged 8-12 years and sexually immature. A valid growth hormone response was recorded in 17 out of 20 healthy children, but in none of the patients. It was concluded that oral administration of L-proline is a valid stimulatory test for increasing serum growth hormone in a clinically reliable manner. It was stated that “the tolerance to L-proline did not differ significantly between the two lots” and that “no adverse effect attributable to proline was recorded in any of the 40 children tested”.

2.4.2 Animal studies

Table 2.4.2-1 summarises the animal studies on safety of L-proline identified in the systematic literature search for the current risk assessment.

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

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Animals</th>
<th>Doses</th>
<th>Outcomes</th>
<th>Duration of exposure</th>
<th>Adverse effects</th>
<th>NOAEL[^1] (mg/kg bw per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schieber et al. (1997)</td>
<td>Sprague-Dawley rats, 7/group, female</td>
<td>0 (control), 50 mg/kg bw per day in drinking water</td>
<td>Urine biochemistry: creatinine, osmomolality Serum biochemistry: Glutamic-oxalic-transaminase, Glutamic-pyruvic-transaminase, alkaline phosphatase, urea, creatinine Organ weights: Liver, left kidney, brain, thymus Histopathology: Left kidney; peripheral and central liver sections</td>
<td>28 days (subacute)</td>
<td>None</td>
<td>Not addressed</td>
</tr>
<tr>
<td>Tada et al. (2010)</td>
<td>Fischer 344 rats, 10/sex/group</td>
<td>0 (control), 0.625%, 1.25%, 2.5%, 5.0% in powder diet</td>
<td>Urine biochemistry, Hematology, Serum biochemistry, Organ weights, Histopathology[^2]</td>
<td>90 days (subchronic)</td>
<td>None</td>
<td>M: 2773 F: 3009</td>
</tr>
</tbody>
</table>

[^1]: Values represent the highest (Tada, 2010) or the only dose tested (Schieber, 1997).
[^2]: A wide range of parameters were monitored and a wide range of organs were examined histopathologically. For details, please confer the original publication.
**Evaluation of D-amino acid levels in rat by gas chromatography-selected ion monitoring mass spectrometry: no evidence for subacute toxicity of orally fed D-proline and D-aspartic acid. Schieber et al., J Chromatogr B Biomed Sci Appl 1997**

This study repeated the design of the older study by Kampel et al. 1990, cited in the IOM (2005) report (see section 2.1.1), feeding L-proline corresponding to 50 mg/kg bw per day through drinking water for 28 days to female Sprague-Dawley rats. In addition to a control group, three other groups with seven animals in each group were fed a corresponding dose of D-proline, D-aspartic acid and L-aspartic acid, respectively.

Feed pellets consisting of grain, coarse soybean meal and 3% fish meal, fortified with 3.8 μmol DL-Met per gram feed and drinking water bottle of 250 ml per cage were offered ad libitum. The control group received deionised water, while the drinking water of the L-proline group was fortified with 0.033% (weight per volume) L-proline. This corresponded to a daily dose of approximately 50 mg L-proline per kg body weight when assuming a daily water consumption of 30 ml and a mean body weight of 200 g through the study period.

Outcome parameters studied were amount of food and water consumed and body weight, serum GOT, GPT, alkaline phosphatase, urea, and creatinine, and urine creatinine and osmolality. On autopsy, fresh weights of liver, left kidney, brain and thymus were determined. Histological examination was performed on sections of the left kidney and two liver sections (peripheral and central), under blinded conditions by two independent observers.

Six of 12 parameters were significantly affected in the rats given L-proline. These included increased food consumption, increased body weight, increased liver weight and GPT, as well as increased urinary creatinine and osmolality. The changes were, however, very small and their biologic significance uncertain. The histological evaluation revealed no pathological changes in kidney or liver.

The authors concluded that no evidence of subacute toxicity was found when feeding rats amino acids corresponding to 50 mg/kg bw per day for 28 days.

**Toxicological evaluation of L-proline in a 90-day feeding study with Fischer 344 rats. Tada et al., Regul Toxicol Pharmacol 2010**

This was a subchronic oral toxicity study in Fischer 344 rats, funded by the Japanese Ministry of Health, Labour and Welfare to provide safety information for natural food additives (Tada et al., 2010). In this study, 10 male and 10 female rats in each group received the following doses L-proline through a cornstarch-based powder diet: 0 (control), 0.625%, 1.25%, 2.5% and 5.0%. According to the authors the study was conducted in correspondence with official guidelines for designation of food additives and for revision of standards for use of food additives by the Japanese Ministry of Health, Labour and Welfare.
Body weight and food and water intake were monitored weekly. At the end of the experiment period, urine and blood samples were collected, and the rats were euthanised. The brain, thyroids (with parathyroids), heart, spleen, liver, adrenal glands, kidneys, testes, ovaries and uterus were excised, and their absolute and relative weights were determined. A wide range of organs were prepared histologically and examined. No endocrine parameters were reported.

For both sexes, average body weights and food intakes throughout the study period did not differ between the control groups and the L-proline treated groups. All treated rats showed no abnormal signs for general appearance, attitude, behaviour or nervous system compared to the control rats during the study. No treatment-related changes were observed in urine parameters including urobilinogen, occult blood, bilirubin, ketone, glucose, protein, pH and nitrous acid.

Concerning hematology, in male rats, hemoglobin concentration at 0.625% and 1.25% L-proline and hematocrit level at 1.25% L-proline were significantly lower than in the control group. In female rats, hemoglobin concentration at 1.25% L-proline and mean corpuscular hemoglobin at 1.25% and 5.0% L-proline were significantly lower than in the control group. The morphological findings and differential counts of leukocytes showed no significant effects in any of the treated groups.

Concerning serum biochemistry, the levels of glucose at 0.625% or higher L-proline in males, blood urea nitrogen at 0.625%, 1.25% and 5.0% L-proline in females, creatinine at 5.0% L-proline in both sexes, uric acid at 2.5% or higher L-proline in males and at 0.625% and 5.0% L-proline in females, and potassium at 5.0% L-proline in males were all significantly lower than in the control groups. On the other hand, calcium at 5.0% L-proline in males and sodium at 5.0% L-proline in females were significantly higher than in the control groups.

Concerning organ weights, in male rats, the relative spleen weights at 2.5% or higher L-proline and the relative kidney weight at 5.0% L-proline were significantly higher than those of the control groups. Although these differences were statistically significant (a high number of comparisons were made using a significance level of 0.05 with no correction for multiple testing), differences in organ weights and all abovementioned biochemical parameters were within the normal physiological range at all dose levels.

Concerning histopathology, no treatment-related macroscopic changes were observed in any organs of either sex, whereas sporadic spontaneous lesions were observed both in the control and the treated animals.

Thus, the highest dose was considered harmless as it produced no functional or morphologic kidney or liver injury, no histopathological changes in the central nervous system were found and no clinical signs of or symptoms suggesting neurotoxicity were observed. The average chemical intakes of L-proline in the 5.0% groups were estimated to be 2773 mg/kg bw per day in the male rats and 3009 mg/kg bw per day in the female rats.
The authors concluded that the NOAEL was "at least a dietary dose of 5.0% (2772.9 mg/kg body weight/day for males and 3009.3 mg/kg body weight/day for females) under the present experimental conditions".

2.4.2.1 Interactions

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

2.4.2.2 Allergic sensitisation (including adjuvant effects)

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

2.4.3 Mode of action for adverse effects

No specific or definite mechanisms for adverse effects have been described.

2.4.4 Vulnerable groups

Patients with hereditary hyperprolenimia (Mitsubuchi et al., 2014) or other rare inborn errors in proline metabolism are considered outside the scope of this risk assessment. No specific vulnerable groups to excess doses of L-proline have been reported. There have been no relevant studies involving elderly, pregnant women or lactating women. Concerning the safety of L-proline in children, VKM has identified one study where a single oral dose of 500 mg/kg bw L-proline was administered with no reports of adverse effects. Any evidence to assume a different tolerance level for L-proline in children or adolescents from that in adults has not been found.

2.5 Summary of hazard identification and characterisation

We are not aware of any randomised controlled trials where humans have been supplemented with L-proline as such, neither in healthy humans nor in any patients groups; neither in adults nor in children or adolescents; neither short-term nor long-term.

One experimental clinical study has been performed in children (Popa et al., 1982), where a single oral dose of L-proline corresponding to 500 mg/kg bw was administered as a growth hormone stimulatory agent to 20 children with hyposomatotropic dwarfism and 20 healthy children (Popa et al., 1982). No adverse effects were observed. There was no randomisation or placebo control in this study.
Previous reports and reviews have cited one study (Hayasaka et al., 1985) where four patients aged between 4 and 32 years with gyrate atrophy of the choroid and retina due to a genetic abnormality received supplementation with between 2 and 10 g/day proline, with or without additional vitamin B₆, for varying duration (up to five years). No overt adverse effects were reported; however, it was uncertain from the paper which effects were studied. This study does not provide any information of value for the current risk assessment.

A few animal model studies have been performed supplementing rats or mice with L-proline, of which one study (Tada et al., 2010) was a subchronic (90 days) dose-response toxicity study in rats. In this study, the highest dose given through a powder diet (5.0% L-proline) produced no growth depression, no functional or morphologic kidney or liver injury, no histopathological changes in the central nervous system and no clinical signs of or symptoms suggesting neurotoxicity. The average exposures of the rats receiving the highest dose were estimated to be 2773 mg/kg bw per day in males and 3009 mg/kg bw per day in females.

No relevant information has been obtained concerning chronic exposure to L-proline in humans nor in animal models.

For the risk characterisation (chapter 4), the NOAEL of 2773 mg/kg bw per day derived from the abovementioned subchronic toxicity study in rats is used for comparison with the estimated exposures from food supplements.
3 Exposure / Intake

Exposure of L-proline 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 50, 500, 1000, 1500 and 1800 mg/day of L-proline 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-proline from specified doses in food supplements to children, adolescents and adults

<table>
<thead>
<tr>
<th>Groups</th>
<th>Daily doses (mg)</th>
<th>Body weight (kg)</th>
<th>Exposures (mg/kg bw per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children (10 to &lt;14 years)</td>
<td>50, 500, 1000, 1500 and 1800</td>
<td>43.4</td>
<td>1.2, 11.5, 23.0, 34.6 and 41.4</td>
</tr>
<tr>
<td>Adolescents (14 to &lt;18 years)</td>
<td>50, 500, 1000, 1500 and 1800</td>
<td>61.3</td>
<td>0.8, 8.2, 16.3, 24.5 and 29.4</td>
</tr>
<tr>
<td>Adults (≥18 years)</td>
<td>50, 500, 1000, 1500 and 1800</td>
<td>70.0</td>
<td>0.7, 7.1, 14.3, 21.4 and 25.7</td>
</tr>
</tbody>
</table>

3.2 Other sources

Based on the NHANES III (1988-1994), the overall mean intake of L-proline from food and food supplements in the United States was 5.2 g/day. The overall 99th percentile was 10.6 g/day. Boys aged 14-18 years had the highest intake at the 99th percentile of 12.0 g/day (IOM, 2005).
4 Risk characterisation

The doses received from NFSA for assessment were 50, 500, 1000, 1500 and 1800 mg/day L-proline in food supplements, and the estimated exposures for adults, adolescents and children 10 years and older derived from these dose levels are given in chapter 3.

The NOAEL used is 2773 mg/kg bw per day.

The margins of exposure (MOE) for the five different doses in the age groups considered, defined as the NOAEL divided by the magnitude of exposure (based on Table 3.1-1) are shown in Table 4-1. The MOE for the highest dose (1800 mg/day) represents a factor of 67 in children (10 to <14 years; default body weight 43.4 kg) and a factor of 94 in adolescents (14 to <18 years; default body weight 61.3 kg). For the dose of 1500 mg/day, the MOE in children is 80. MOE for all other dose and age categories are above 100.

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>50 mg/ day</th>
<th>500 mg/ day</th>
<th>1000 mg/ day</th>
<th>1500 mg/ day</th>
<th>1800 mg/ day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children (10 to &lt;14 years) (43.4 kg)</td>
<td>2407</td>
<td>241</td>
<td>120</td>
<td>80</td>
<td>67</td>
</tr>
<tr>
<td>Adolescents (14 to &lt;18 years) (61.3 kg)</td>
<td>3400</td>
<td>340</td>
<td>170</td>
<td>113</td>
<td>94</td>
</tr>
<tr>
<td>Adults (≥18 years) (70 kg)</td>
<td>3882</td>
<td>388</td>
<td>194</td>
<td>129</td>
<td>108</td>
</tr>
</tbody>
</table>

Based on the magnitude of the margins of exposure, the lack of adverse effects at the highest dose tested (current NOAEL) and the notion that L-proline is a nutrient that is produced endogenously in addition to being consumed at a mean intake in the magnitude of 5 grams per day, VKM considers that L-proline at the specified doses 50, 500, 1000, 1500 and 1800 mg/day are unlikely to cause adverse health effects in all age groups covered by the current risk assessment.
5 Uncertainties

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

- The lack of relevant human data available for risk characterisation
- The risk characterisation being based on a 90-day (subchronic) study in rats, yielding uncertainty related to:
  - the lack of knowledge about potential risks related to chronic exposure
  - extrapolation to humans (an uncertainty factor of 10 was considered acceptable)
  - interindividual variation (a factor of at least 6.7 was considered acceptable)
- The lack of knowledge about habitual daily dietary intake of L-proline in Norway as well as the magnitude of daily endogenous synthesis of L-proline, both in which there may be large interindividual variations
- The assumption that children and adolescents have similar tolerance as adults relative to their body weight, due to lack of data
- The use of default body weights for the three respective population groups determined by EFSA for estimation of average exposure to L-proline per kg body weight from the designated dose levels. With use of the default average body weight of an age group, the interindividual variance in the group is not taken into account, and individuals with body weights lower than the default body weight are thus not fully covered in the risk estimate
- The possible failure of the systematic literature search, based on the predefined search criteria, to identify relevant literature reporting adverse effects of L-proline in humans or animals
6 Conclusions with answers to the terms of reference

The Norwegian Food Safety Authority (NFSA) requested the Norwegian Scientific Committee for Food Safety (VKM) to assess the safety of L-proline in food supplements at the doses 50, 500, 1000, 1500 and 1800 mg/day for the general population, ages 10 years and above.

VKM concludes that:

- In adults (≥18 years), the specified doses 50, 500, 1000, 1500 and 1800 mg/day L-proline in food supplements are unlikely to cause adverse health effects.
- In adolescents (14 to <18 years), the specified doses 50, 500, 1000, 1500 and 1800 mg/day L-proline in food supplements are unlikely to cause adverse health effects.
- In children (10 to <14 years), the specified doses 50, 500, 1000, 1500 and 1800 mg/day L-proline 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-proline in food supplements.

Green: Estimated exposures to L-proline are unlikely to cause adverse health effects.

<table>
<thead>
<tr>
<th>Age groups</th>
<th>L-proline Doses</th>
<th>50 mg/day</th>
<th>500 mg/day</th>
<th>1000 mg/day</th>
<th>1500 mg/day</th>
<th>1800 mg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children (10 to &lt;14 years)</td>
<td></td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
</tr>
<tr>
<td>Adolescents (14 to &lt;18 years)</td>
<td></td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
</tr>
<tr>
<td>Adults (≥18 years)</td>
<td></td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
</tr>
</tbody>
</table>
7 Data gaps

We are not aware of any randomised placebo-controlled trials where humans have been supplemented with L-proline as such, neither in healthy humans nor in any patients groups; neither in adults nor in children or adolescents; neither short-term nor long-term.
8 References


FVM. (2014) Bekentgørelse om tilsætning af visse andre stoffer end vitaminer og mineraler til fødevarer, Fødevareministeriet (FVM), Fødevarestyrelsen, Denmark.


IOM. (2005) Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids, Institute of Medicine, Washington DC.


Appendix 1

Search strategies for this risk assessment

Search strategy for 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. proline*.ti. (15090)
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. (9789711)
3. 1 and 2 (2760)
4. (conference abstract* or letter* or editorial*).pt. (4934471)
5. 3 not 4 (2655)
6. limit 5 to (danish or english or norwegian or swedish) (2643)
7. limit 6 to human (929)
8. remove duplicates from 7 (586)

Search strategy for animal studies

Database: Embase <1974 to 2016 August 02>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present>

1. proline*.ti. (15288)
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. (10139037)
3. 1 and 2 (2808)
4. (conference abstract* or letter* or editorial*).pt. (5121834)
5. 3 not 4 (2701)
6. limit 5 to (danish or english or norwegian or swedish) (2689)
7. limit 6 to animals (749)
8. remove duplicates from 7 (542)

Search strategy for studies in children and adolescents

Database: Embase <1974 to 2016 October 25>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present>

1. proline*.ti. (15422)
2. (child* or adolescent* or teenage* or college* or high school).tw. (3101342)
3. 1 and 2 (86)
4. limit 3 to (danish or english or norwegian or swedish) (76)
5. remove duplicates from 4 (51)