Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

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

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Assessed by

The opinion has been evaluated and approved by the Panel on nutrition, dietetic products, novel food and allergy of VKM.

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Summary

In 2011, the Norwegian Scientific Committee for Food Safety (VKM) conducted a risk categorisation of 30 amino acids and amino acid compounds. Based on potential health risks related to high intakes of the amino acids they were categorised into low, moderate or high risk groups. The amino acids histidine, methionine, S-adenosylmethionine (SAM) and tryptophan were categorised into the high risk group in this first screening.

Based on the risk categorisation, the Norwegian Food Safety Authority has requested VKM to conduct a risk assessment of the four amino acids histidine, methionine, SAM and tryptophan added to foods and drinks and in food supplements. This opinion is limited to the use of single free amino acids in food supplements or fortified foods and drinks, and does not elaborate on risks related to protein hydrolysates or high protein intake.

This opinion has been prepared, evaluated and approved by the VKM Panel on Nutrition, Dietetic products, Novel Food and Allergy.

The current opinion is based on conclusions from *Dietary Reference Intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids* the US Institute of Medicine (IOM, 2005) and literature identified in a literature search conducted in October 2012. In their report IOM concluded that there were insufficient data available to evaluate the safety of single free amino acids and that no tolerable upper intake levels (UL) could be established.

Histidine, methionine and tryptophan are essential amino acids, whereas SAM is a metabolite from methionine. Mild adverse effects such as nausea and reduced appetite were reported with the use of all four amino acids. There are indications that intake of methionine during the so called methionine-loading test (100 mg methionine per kg body weight) is associated with adverse health effects such as dizziness, sleepiness and decreased or increased blood pressure. Intake of methionine supplement seems to be a concern for health also because increased concentration of its metabolite homocysteine in plasma may be associated with cardiovascular disease.

Intake of tryptophan supplement (single dose 6 g) has resulted in a significant increase in lipid peroxidation products, indicating an increased oxidative stress level. Intake of tryptophan supplement (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.

Because no dose-response studies or adverse health effects related to dose were found, UL for these four amino acids could not be established. However, in this assessment a tentative guidance level (GL) at 210 mg/day is suggested for methionine, and 220 mg/day is suggested as a tentative daily GL for tryptophan.

The intake of these four amino acids in free form was based on the doses used in food supplements sold in Norway, VKM estimates the intake of free form histidine and SAM to be within an acceptable level, whereas the doses of methionine in supplements available at the Norwegian market is higher than the tentative suggested GL in this assessment. According to information from the Norwegian Food Safety Authority, supplements with single free tryptophan are not sold on the Norwegian market.

Norsk sammendrag

I 2011 gjennomførte Vitenskapskomiteen for mattrygghet (VKM) en risikokategorisering av 30 aminosyrer og aminosyreforbindelser. Basert på potensiell helserisiko knyttet til høyt inntak av aminosyrene ble de kategorisert i lav, moderat eller høy risikogruppe. Aminosyrene histidin, metionin, S-adenosylmetionin (SAM) og tryptofan ble kategorisert i høy risikogruppe i denne første screeningen. Basert på denne risikokategoriseringen, har Mattilsynet bedt VKM om å foreta en risikovurdering av de fire aminosyrer histidin, metionin, SAM og tryptofan tilsatt i mat eller drikke og i kosttilskudd.

Denne vurderingen er begrenset til bruk av frie aminosyrer i kosttilskudd eller beriket mat og drikke, og omfatter ikke hydrolyserte proteiner eller generelt høyt proteininntak. Vurderingen er utarbeidet, evaluert og godkjent av VKMs Faggruppe for ernæring, dietetiske produkter, ny mat og allergi.

Denne risikovurdering er basert på konklusjoner i *Dietary Reference Intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids* fra Institute of Medicine i USA (IOM, 2005) og litteratur identifisert i litteratursøk gjennomført i oktober 2012. I sin rapport konkluderte IOM med at det ikke var tilstrekkelige data til å vurdere sikkerheten ved bruk av disse frie aminosyrene og at det ikke kunne fastsettes et tolerabelt øvre inntaksnivå.

Histidin, metionin og tryptofan er essensielle aminosyrer, mens SAM er en metabolitt fra metionin. Milde bivirkninger som kvalme og redusert appetitt er rapportert ved bruk av alle de fire aminosyrene. Det er indikasjoner på at inntak av metionin under såkalt metionin-loading test (100 mg metionin per kg kroppsvekt) er forbundet med negative helseeffekter som svimmelhet, søvnighet og redusert eller økt blodtrykk. Inntak av metionintilskudd synes å kunne medføre økt helserisiko også fordi økte konsentrasjoner av metabolitten homocystein i plasma kan være forbundet med økt risiko for hjerte- og karsykdom.

Inntak av tryptofantilskudd (enkeltdose 6 g) har resultert i signifikant økt lipidperoksidering - noe som indikerer økt oksidativt stressnivå. Inntak av tryptofantilskudd (4,2 g/dag) har vært knyttet til utviklingen av eosinofili, men dette spørsmålet er fortsatt uavklart. Inntil dette er helt avklart bør tryptofantilskudd fortsatt anses å kunne medføre økt helserisiko.

Fordi dose-respons studier mangler eller negative helseeffekter ikke kan knyttes til konkrete doser, kan det ikke fastsettes UL for disse fire aminosyrene. Imidlertid foreslår Faggruppen for ernæring, dietetiske produkter, ny mat og allergi tentative Guidance Levels (GL) på 210 mg/dag for metionin, og 220 mg/dag for tryptofan.

Inntak av disse fire aminosyrer i fri form er kun vurdert ut fra de dosene som forekommer i kosttilskudd som selges i Norge i dag (opplyst av Mattilsynet). På bakgrunn av dette anser VKM at inntaket av fri form histidin og SAM ligger innenfor akseptable nivåer, mens doser av metionin i kosttilskudd tilgjengelig på norske markedet er høyere enn den tentative foreslått GL i denne vurderingen. Mattilsynet har ikke opplysninger om at kosttilskudd med fri form tryptofan er i salg på det norske markedet.

Contents

Background

Amino acids are added to various foods and drinks such as sports products, food supplements and soft drinks/energy drinks. Regulation of amino acids fortification is not harmonised in the EU. In Norway, amino acid fortification must be approved according to §6 in the national regulation 26 February 2010 no. 247 on addition of vitamins, minerals and certain other substances.

In 2011, The Norwegian Scientific Committee for Food Safety (VKM) conducted a risk screening of 42 amino acids and amino acid compounds. Based on available literature, the amino acids and amino acid compounds were divided into three groups; potential low, moderate or high risk group. Amino acids that in any human or animal study or case report etc. indicated any direct harmful influence on organs, effect on nervous system or have a potential to increase the risk for a disease were categorised in the potential high risk group. It must be emphasised that the screening was not a full risk assessment.

Based on this risk screening from 2011, the Norwegian Food Safety Authority (Mattilsynet) has requested VKM to conduct a risk assessment of four amino acids; histidine, methionine, S-adenosylmethionine (SAM) and tryptophan. These amino acids are already available in food supplements, amino acid drinks or amino acid/protein powders on the Norwegian market.

This opinion has been prepared by Margaretha Haugen, Jutta Dierkes, Azam Manzoor and Bjørn Skålhegg and evaluated and approved by the Panel on Nutrition, Dietetic products, Novel Food and Allergy in VKM.

Terms of reference

The Norwegian Food Safety Authority has requested the Norwegian Scientific Committee for Food Safety (VKM) to conduct a risk assessment of the amino acids histidine, methionine, S-adenosylmethionine and tryptophan added to foods and drinks and in food supplements.

The assessment should answer the following questions:

- What are the negative health effects of these four amino acids?
- Are there any special population groups that should avoid one or more of these four amino acids?
- Can an upper tolerable intake level be set for these amino acids?
- What is the population intake of these four amino acids added to foods and drinks and in food supplements?

Assessment

This assessment includes an evaluation of added free single amino acids. Amino acids as part of proteins in regular diets or as partially or extensively hydrolysed good-quality proteins have a long history of safe use and so far no negative health effects have been reported (Schaafsma, 2009). According to the Nordic Nutritional Recommendations (NNR) 2004 an intake of protein two to three times above the recommended intake has been associated with increased risk of juvenile diabetes, and adiposity in children, increased calcium losses and homocysteinemia. However, NNR conclude that in general very high intake of protein cannot be recommended, but does not seem to pose health concerns. Single amino acids are used because they have biological effects by themselves or as precursors to other compounds with biological effects, and intake of single amino acids may be of concern (IOM, 2005). Intake of single amino acid will enter the circulation faster than amino acids bound in protein and hydrolysates and if the net protein capacity is insufficient to compensate for the increased entry, amino-acid oxidative pathway will be stimulated to keep the plasma amino acid concentration within an acceptable safe range (Erlandsen et al., 2003). The pool of free amino acids in the body is very small and an imbalance between the 22 amino acids constituting human protein could also impair protein synthesis (Erlandsen et al., 2003).

General intake calculations of the amino acids could not be performed as The Norwegian Food Composition Table does not include information about amino acid content.

1 Previous risk assessments of amino acids

In 2002, the US Institute of Medicine (IOM) made an effort to establish tolerable upper intake levels for the individual amino acids, and attention was diverted towards foods and drinks with added amino acids and food supplements (IOM, 2005). It was concluded that insufficient data were available to evaluate the safety of single free amino acids and no tolerable upper intake levels could be established.

Histidine

IOM concludes that doses of L-histidine between 4 and 4.5 g/day in addition to the amounts found in the diet, do not result in adverse effects (IOM, 2005). However, IOM argues that this evidence should be considered tentative given the few individuals studied and lack of dose–response information. Furthermore, IOM states that there is evidence from studies in experimental animals and humans that intakes of high levels of histidine can alter copper and zinc metabolism. However, the lack of dose–response data precludes identifying the intake concentrations in humans required to elicit such responses. According to IOM, the available scientific data are not adequate to derive a UL for the chronic oral intake of L-histidine from supplements (IOM, 2005).

Methionine

IOM reports that dietary excesses of L-methionine (2.7% of the diet) for 6, 13, or 20 days have been associated with erythrocyte engorgement and accumulation of hemosiderine in rats, and splenic damage and a reduced growth rate (Benevenga, 1976 in IOM, 2005). A single dietary dose (2.7% of the diet) of L-methionine decreased body growth and also reduced food intake in rats (Steele et al., 1979 in IOM, 2005). Dietary intakes of 2-4% of L-methionine caused slight changes in liver cells in rats (Stekol and Szaran, 1962 in IOM, 2005) and slight

decreases in liver iron content (Klavins et al., 1963 in IOM, 2005). Darkened spleens caused by increased iron deposition have been observed in rats fed 1.8% methionine diets for 28 days (Celander and George, 1963 in IOM, 2005). Subnormal foetal and placental weights were reported in pregnant rats fed 4% of their diet as methionine (Viau and Leathem, 1973 in IOM, 2005). However, supplemental methionine prevented neural tube defects in rat embryos treated with teratogenic antivisceral yolk sac serum (Fawcett et al., 2000 in IOM, 2005). In the mouse, the administration of methionine reduced experimentally induced spina bifida (Ehlers et al., 1994 in IOM, 2005). Other studies in rodent and primate models support the beneficial effect of methionine supplementation in improving pregnancy outcomes (Chambers et al., 1995; Chatot et al., 1984; Coelho and Klein, 1990; Ferrari et al., 1994; Moephuli et al., 1997 all in IOM, 2005).

In humans, single oral doses of about 0.6 g (adults) and 0.08 g (infants) led to increased plasma levels of L-methionine and L-alanine, and decreased plasma concentrations of leucine, isoleucine, valine, tyrosine, tryptophan, and phenylalanine (Stegink et al., 1980, 1982b in IOM, 2005). None of these studies report any adverse health effects. Methionine supplements (5 g/day) for weeks were reportedly innocuous in humans (Health and Welfare Canada, 1990 in IOM, 2005). A single oral dose of 7 g has been associated with increased concentration of mixed disulfides (Brattstrom et al., 1984 in IOM, 2005). Single oral methionine doses at 7 g produced lethargy in six individuals, and oral administration of 10.5 g of L-methionine to one individual caused nausea and vomiting (Perry et al., 1965 in IOM, 2005). After oral doses of 8 g/day of methionine (isomer not specified) for four days, serum folate concentrations were decreased in five otherwise healthy adults (Connor et al., 1978 in IOM, 2005). High doses of methionine (~100 mg/kg body weight) led to elevated plasma methionine and homocysteine concentrations (Brattstrom et al., 1984, 1990; Clarke et al., 1991; Wilcken et al., 1983 all in IOM, 2005). According to the IOM report it was suggested that elevated plasma homocysteine concentrations may be a risk factor for coronary disease (Clarke et al., 1991 in IOM, 2005).

Infants metabolise methionine more rapidly than adults (Stegink et al., 1982b in IOM, 2005). In women whose average daily intake of methionine was above the lowest quartile of intake (greater than 1.34 g/day), a 30 to 40% reduction in neural tube defect-affected pregnancies was observed (Shaw et al., 1997 in IOM, 2005). These reductions were observed for both anencephaly and spina bifida.

According to IOM, a dose-response relationship for methionine could not be identified due to lack of adequate data. Thus the data on the adverse effects of L-methionine from supplements were considered insufficient to establish a UL for apparently healthy humans (IOM, 2005).

Tryptophan

In1991, Funk et al. found that rats given a 20% casein diet supplemented with 14.3% tryptophan for four weeks developed scaly tails and thinning of hair (Funk et al., 1991 in IOM, 2005). No adverse effects were seen when the diet was supplemented with 1.4 or 2.9% L-tryptophan. No cancers were observed over an 80-weeks period when rats were fed diets with added 2% L-tryptophan (Birt et al., 1987 in IOM, 2005). Maternal rats' diets supplemented with 1.4 and 6% L-tryptophan have resulted in impaired weight gain and foetal weight (Funk et al., 1991 and Matsueda and Niiyama, 1982, both in IOM, 2005).

In humans, administration of 0, 1, 2 and 3 g of L-tryptophan has resulted in decreased hunger and alertness and increased faintness and dizziness (Hrboticky et al., 1985 in IOM, 2005). Ten healthy adults given 5 g of L-tryptophan in a double-blind, placebo-controlled study reported

severe nausea and headache and increased drowsiness soon after ingestion (Greenwood et al., 1975 in IOM, 2005). Several other studies report drowsiness, increased fatigue and prolonged lethargy in adults with intakes of L-tryptophan in single doses ranging from 50-100 mg/kg body weight (IOM, 2005).

IOM reports that Blauvelt and Falanga (1991) examined the history of L-tryptophan use in 49 patients with cutaneous fibrosis. Eleven of 17 patients reported using L-tryptophan prior to onset of eosinophilic fasciitis, and so did two of ten patients with localised scleroderma. Use of L-tryptophan was not reported in any of 22 patients with systemic sclerosis. Intakes of L-tryptophan were from 0.5 to 5 g/day for one month to 10 years before the onset of eosinophlic fasciitis was noted. Intakes of L-tryptophan were from 1.5 to 2 g/day for three or 10 months before onset of symptoms of localised scleroderma occurred. Furthermore, IOM reports that Hibbs and coworkers (1992) found that nine of 45 patients with eosinophilic fasciitis used 0.5 to 2.5 g/day of L-tryptophan for one month to 10 years before symptom onset. It is unknown whether or not these results occurred because of impurities in the L-tryptophan supplements (IOM, 2005).

IOM concludes that studies in humans indicate that relatively short-term (acute and subacute) use of L-tryptophan is associated with appetite suppression, nausea, and drowsiness. 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 UL could not be established for L-tryptophan (IOM, 2005).

From animal studies reviewed in Moehn et al., 2012, it appears that high tryptophan intakes result in reduced feed intake and growth rate. In growing animals, however, tryptophan intakes of >10 times the requirement are necessary before there are detrimental effects on growth performance. At still higher intakes, fatty liver and fibrotic changes in muscles, lung, and pancreas and the serotonin syndrome may develop (Moehn et al., 2012).

In 2006, the Committee on Toxicity in UK Food Standard Agency maintained the opinion that the current UK regulations should remain in place, allowing the use of tryptophan as a food supplement at a daily dose up to 220 mg[1].

2 Literature search

This chapter describes the literature search conducted for retrieving the scientific documentation available for the sections 3.2, 3.3, 4.2, 4.3, 5.2, 5.3, 6.2 and 6.3.

2.1 Search strategy

The main literature searches for each of the four amino acids were conducted in collaboration with a librarian. Test searches were conducted to find relevant terms and search words, and controlled vocabulary (MeSH and EMTREE) in core articles was examined.

Search strategies were then developed and literature searches conducted in Medline and Embase. The searches were conducted in October 2012. The literature search string for

[1]http://tna.europarchive.org/20120209120540/http:/www.food.gov.uk/news/newsarchive/2006/mar/tryptophanupdate?view=printerfriendly

tryptophan, histidine, methionine and S-adenosylmethionine (SAM) is described in detail in Annex 1.

In the case of S-adenosylmethionine, an additional search was conducted with the following search words: S-adenosylmethionine AND stereoisomers, S-adenosylmethionine AND purity, S-adenosylmethionine AND stability. Three studies that were relevant to toxicity were identified in this additional search.

If references deemed relevant were discovered which had not appeared in the search, these were included.

As IOM published risk assessments of the amino acids in 2005, the search period covered publications from 2002 onwards unless substantial contributions, to our understanding, had been published before this date and not included in the IOM assessment. SAM is not included in the IOM risk assessment, and the search period for SAM is therefore not limited from 2002 onwards. Articles in English and Scandinavian languages were included in the search.

2.2 Study designs

Human and animal studies are included, and *in vitro* studies are excluded.

2.3 Publication selection

Initially, the titles and the abstracts of all papers identified in the search process were independently assessed for relevance by two reviewers. Articles were excluded if they did not include information on one of the four amino acids; tryptophan, histidine, methionine or SAM in addition to a negative or adverse health effect. Literature such as doctoral thesis, conference proceedings and reports were excluded.

In the next step, full text articles were assessed independently by two reviewers, and papers were excluded if they did not address adverse health effects.

3 Histidine

3.1 Identification and characterisation of histidine

Molecular name is L-histidine and molecular weight is 155.157 g/mol.

Drugbank[2] ID: DB00117

L-histidine is a basic, genetically coded amino acid. Although histidine is generally regarded as an indispensable (essential) amino acid, removal of histidine from the diet does not induce negative nitrogen balance in the first 10 days. Nitrogen balance becomes gradually negative after a longer period of histidine withdrawal and nitrogen balance rapidly becomes positive after reintroduction of histidine (Kopple and Swendseid, 1975).

Histidine is an important component of hemoglobin (8%), with the bulk being in the globin portion. The hemoglobin concentration falls in adults on a histidine-free diet and is reversed when histidine is restored (Kopple and Swendseid, 1975). Moreover, the dipeptide carnosine,

[2] http://www.drugbank.ca.

found in skeletal muscle serves as a body pool of histidine (Christman, 1971). Because of large body pools of histidine it takes a prolonged period (more than 60 days) to deplete an adult of histidine. Histidine is the precursor for histamine biosynthesis.

Daily nutritional requirement for histidine is estimated to be 33 mg/kg in infants (adults not known) (IOM, 2005).

3.2 Animal and human studies with histidine

No new animal toxicity studies or human studies with histidine reporting adverse health effects were identified in the literature search (after 2002).

3.3 Exposure assessment

Red meat, poultry, fish and dairy products are rich histidine sources. Soy contains histidine, but generally plants are not as important sources as animal products. Based on distribution data from the 1984-1994 NHANES III, the mean daily intake of histidine for all life stage and gender groups of from foods and supplements is 2.2 g/day (IOM, 2005).

According to the Norwegian Food Safety Authority, there are amino acid powders available on the Norwegian market that contain up to 2.5 g histidine per recommended daily dosage.

3.4 Special groups

No special groups identified.

3.5 Summary histidine

Histidine is an essential amino acid in humans but in adults histidine is stored as carnosine in muscles, and depletion might take several months. In contrast, immediate negative nitrogen balance is reported in infants on a histidine free diet because of minimal histidine stores. In 2005, IOM concluded that it was insufficient data to establish a UL for histidine, but stated that doses at 4 to 4.5 g/day have not resulted in adverse effects. No relevant new animal or human studies with histidine were identified after 2002.

3.6 Discussion/conclusion

There are few studies after 2000 which describe research on histidine, and none describing adverse health effects of histidine supplementation. In our risk grouping of amino acids in 2011, histidine was assigned a high risk amino acid because of reported adverse effects on the eye at high doses (64 g L-histidine /day) (Geliebter et al., 1981). The authors also reported adverse effects like headache, weakness, drowsiness and mental confusion. The main concern with L-histidine is the findings that L-histidine can alter copper and zinc metabolism, and this concern has still not been clarified.

Because no relevant new animal or human studies with histidine were identified after 2002 there is no new evidence for suggesting a UL. The Panel on nutrition, dietetic products, novel food and allergy supports the IOM conclusion that doses at 4 to 4.5 g/day have not resulted in adverse effects.

No special population groups have been identified with concern to L-histidine supplementation.

Amino acid supplements sold in Norway containing 77 mg to 2.5 g L-histidine per daily dosage should not be of concern.

4 Methionine

4.1 Identification and characterisation of methionine

Molecular name is L-methionine and molecular weight is 149.21 g/mol.

Drugbank[3] ID: DB00134

Methionine is an essential amino acid which is converted into S-adenosylmethionine (SAM) by the enzyme methionine adenosyltransferase, which has nearly 80% activity located in the liver. In this reaction adenosyl triphosphate (ATP) donates its adenosyl group to methionine and forms inorganic phosphate (P_i) and pyrophosphate (PP_i). SAM donates its methyl group to methyl acceptors (DNA, RNA, proteins and amino acids) and forms S-adenosylhomocysteine. Hydrolysis of S-adenosylhomocysteine generates homocysteine and adenosine.

Decreased intake of methionine decreases the formation of SAM, whereas increased intake of methionine increases the concentration of SAM. Elevation of SAM inhibits the activity of methylenetetrahydrofolate reductase, leading to reduced concentration of serum folate (Finkelstein, 1990; Mansoor et al., 1997; Maron and Loscalzo, 2009).

Methionine metabolism can be divided into L-methionine-transmethylation (TM) and L-methionine-sulfoxidation and transamination (TA) pathways (Dever and Elfarra, 2010). *In vitro* and animal studies suggest that metabolites S-adenosylmethionine (SAM) and cysteine in TM pathway play important protective role in the body. SAM is an important methyl donor and cysteine is a component of glutathione, an essential antioxidant in the body. It has been reported that supplementing cofactor serine in TM pathway will increase the formation of cystathionine from homocysteine. Therefore, in an efficient TM pathway, accumulation of methionine will not take place even at slightly higher methionine supplies.

Contrary to TM pathway, intermediate products of transamination (TA) pathway are toxic and their concentrations increase in individuals with increased plasma concentrations of methionine (hypermethionemia). It has been suggested that various transaminases may be involved in the production of toxic mixed disulfides in this pathway. The individuals with normal physiological concentrations of methionine do not produce toxic products or may have very low concentration of these species (Duescher et al., 1994). The authors suggest that sulfoxidation of L-methionine may take place by free radicals, which are produced by flavin-containing monooxygenases.

Individuals with hypermethioninemia have higher concentration of methionine sulfoxide than healthy subjects.

Even though, hypermethionemia is associated with production of toxic metabolites, it remains to be established how methionine metabolic pathways interact with each other during

[3] http://www.drugbank.ca.

methionine-loading test. Therefore, sophisticated techniques are required to measure methionine metabolites in the serum in patients with hypermethionemia.

4.2 Animal studies with methionine

After 2002, no methionine toxicity studies in animals were identified in the literature. However, review based on animal studies was identified (Dever and Elfarra, 2010), and one study identified in this review was published after 2002 and used in the present opinion (Toue et al., 2006).

In this study, the authors investigated changes in biochemical and hematological parameters in five week old male Fischer rats fed with increasing amount of L-methionine (Toue et al., 2006). Nine rats per group were given L-methionine 0, 0.3, 0.6, 1.2 and 2.4% by weight of diet for 14 days. The rats fed with 2.4% L-methionine by weight of diet showed significant differences in clinical chemistry and hematology parameters compared with the group of mice given 0% L-methionine by weight of diet. It was demonstrated, in a multivariate analysis, that homocysteine and cystathionine were the most prominent parameters, which could differentiate between toxic or non-toxic doses of methionine. Therefore, the authors suggested that the concentration of plasma homocysteine might be used as a biomarker for the upper limit of dietary methionine intake.

4.3 Human studies with methionine

The literature search identified three human studies investigating methionine loading or increased methionine intake published after 2002. Two studies were based on methionine - loading tests in patients and healthy subjects, respectively (Krupkova-Meixnerova et al., 2002; O'Dochartaigh et al., 2004) and one case report (Cottington et al., 2002; Toue et al., 2006). Furthermore, one relevant review was identified (Garlick, 2006), and all references cited in this review (after 2002) were evaluated for inclusion. One additional relevant study in infants was identified (Harvey et al., 2003).

Methionine-loading test has been applied to detect hyperhomocysteinemia in patients with cardiovascular disease. In one study, 296 patients and 591 controls were recruited to investigate the prevalence of hyperhomocysteinemia and genetic markers linked to arteriosclerosis (Krupkova-Meixnerova et al., 2002). The median age of the patients and controls was 56 years and 50 years respectively. In the patient group 82% were men, 55% had hypertension and 24% had diabetes whereas in the control group 48% were men, 13% had hypertension and 4% had diabetes.

Blood samples were collected from all subjects after a 12 hours fast. They were given methionine (100 mg/kg body weight) dissolved in 300-500 ml fruit juice. Blood samples were collected again six hours after methionine-loading. The authors recorded that about 13% subjects complained about dizziness, sleepiness 4%, nausea 3% and change in blood pressure about 3%. Two subjects were admitted to the hospital for serious complications. After clinical and laboratory investigations, it was concluded that methionine-loading might not be a cause for worsening of their illness.

The symptoms of dizziness appeared within one to two hours, ranging between 30 minutes to four hours. The subjects with symptoms of dizziness were examined further for detailed neurological function, which did not show any signs of gross cerebellar dysfunction. The

condition of dizziness was not different between the individuals taking angiotension converting enzyme (ACE) inhibitors or those who were not taking ACE inhibitors.

The authors did not observe complications related to cardiovascular diseases or increased mortality rate in subjects after thirty days of methionine-loading test, therefore it was suggested that methionine-loading test was not associated with increased mortality (Krupkova-Meixnerova et al., 2002).

O´Dochartaigh *et al*., studied parameters of pulmonary vascular function during an exercise test in ten non-smoking, healthy men during methionine-loading (O'Dochartaigh et al., 2004). The mean age of the participants was 27.4 years (range 23-31). At two different occasions, they were given 100 mg methionine/kg body weight, dissolved in fruit juice, or placebo. The concentration of plasma total homocysteine increased significantly after four or six hours of methionine-loading ($p<0.0001$) but did not increase after placebo. There was a significant increase in ventilatory equivalent for CO_2 (V_E/V_{CO_2}) at anaerobic threshold ($p=0.0009$) and a significant decrease in end-tidal partial pressure (P_{ETCO_2}) both at anabolic threshold and at peak exercise during methionine-loading ($p=0.0003$, $p=0.006$ respectively). The authors suggested that methionine-loading might affect respiratory gas exchange system due to pulmonary vascular endothelial dysfunction.

In a case study, Cottington et al., report death of a 69-year old woman after an oral methionine-loading test (Cottington et al., 2002). The woman was taking diltiazem hydrochloride, hydrochlorothiazide, potassium, aspirin and rotecoxib on the daily basis. Her blood pressure on the day of methionine-loading test was 186/77 mm Hg. She started vomiting after the methionine-loading test and seven hours after the test she was confused and agitated. Her blood pressure was elevated to 261/99 mm Hg. Later, she became pulse less and was diagnosed with aspiration pneumonia. She developed transient anemia. The woman died in the hospital 30 days after the methionine-loading test.

Blood samples collected before her death showed an increase in the concentrations of methionine, from 19 to 5760 µmol/L, SAM from 79 to 1089 nmol/L and plasma total homocysteine from nine to 43 µmol/L. The increase in the concentration of methionine, SAM and total homocysteine in plasma was significantly higher than increase in these parameters in healthy individuals after a regular methionine-loading test. The authors suspect that the woman who should have been given 8.39 g methionine was given 80 g methionine, an overdose of methionine (Cottington et al., 2002).

Harvey et al. (2003), describe the possible causes of hypermethioninemia and hyperhomocysteinemia in ten infants. The highest concentrations of plasma methionine and plasma homocysteine measured in these infants were 6830 µmol/L and 44 µmol/L, respectively. In healthy subjects, the reference range for plasma methionine is 13-43 µmol/L whereas the reference range for plasma homocysteine is 5-14 µmol/L. The infants also had high concentrations of plasma adenosylmethionine, adenosylhomocysteine, cystathionine and N-methylglycine. The dietary history of these infants revealed that nine out of ten were given a protein hydrolysate formula in which the content of methionine had been increased up to 8800 mg/L. It was concluded that hypermethioninemia in these babies was a result of increased methionine intake (Harvey et al., 2003).

A brain MRI examination at the time of extreme hypermethioninemia indicated abnormalities related to cerebral oedema in two babies. The authors suggested that methionine or metabolites of methionine during hypermethioninemia might have inhibited the activity of Na^+, K^+-ATPase, which caused cerebral oedema. The authors reported that the anomalies

related to cerebral and metabolic functions were not registered in these babies when the intake of methionine was reduced (Harvey et al., 2003).

4.4 Exposure assessment

High levels of methionine can be found in egg, fish, dairy products, nuts and sesame seeds. Methionine is also found in meat, cereal grains and some other plant seeds. Most fruits and vegetables included legumes are poor methionine sources. Average methionine intake in all age groups from foods and supplements is 1.8 g/day (IOM, 2005).

According to the Norwegian Food Safety Authority, there are supplements available on the Norwegian market that contain up to 500 mg methionine per recommended daily dosage.

4.5 Special groups

The number of patients with the deficiency of cystathionine β-synthase (homozygote form), may be 1:100000 in Europe (Mudd et al., 1985; Mudd et al., 1995). Cystathionine β-synthase plays a pivotal role in mammalian sulfur metabolism and in the conversion of methionine to cysteine via homocysteine. This transsulfuration pathway is the only pathway capable of removing sulfur-containing amino acids under conditions of abundant intake (Finkelstein, 1998). Children with the deficiency of cystathionine β-synthase are usually identified by health personnel in their childhood.

Patient groups with hyperhomocysteinemia should be advised against use of methionine supplementation, because of possible increased risk of coronary vascular disease.

4.6 Summary methionine

Methionine is an essential amino acid, which forms S-adenosylmethionine (SAM), a pivotal methyl donor in the cell. S-adenosylhomocysteine is derived from SAM. Hydrolysis of S-adenosylhomocystein produces homocysteine and adenosine. Homocysteine is a toxic amino acid, which is not stored in the cell but is released in the blood plasma. Increased concentration of plasma homocysteine seems to be a risk factor for coronary vascular diseases.

In 2005, IOM concluded that it was insufficient data to establish a UL for methionine. One relevant new animal and four human studies with methionine were identified after 2002. Two of the new studies in humans reported on methionine-loading tests. One study in infants showed serious adverse health effects in infants given a protein hydrolysate with L-methionine equivalent to 8800 mg/L.

There are indications that intake of methionine during the so called acute methionine-loading test is associated with adverse health effects such as dizziness, nausea, sleepiness and decreased or increased blood pressure. In the loading test, 100 mg methionine per kg body weight is given after a 12-hour fast. This intake (100 mg/kg body weight) of L-methionine may be regarded as the lowest observed adverse effect level (LOAEL).

4.7 Discussion/conclusion

Although IOM has concluded that no UL could be established for methionine it has been reported that use of methionine as a single amino acid may have adverse health effects. An intake at 100 mg/kg body weight of L-methionine may be regarded as a LOAEL. With a conservative approach and the use of an uncertainty factor of 10 for between people variations and a factor of 3 for the uncertainty of LOAEL, a tentative GL of 100/30 ~ 3 mg of L-methionine per kg body weight can be suggested. In a 70 kg man this is equivalent to an intake of 210 mg per daily dosage, which is lower than what is found on the Norwegian market today.

5 S-adenosylmethionine (SAM)

5.1 Identification and characterisation of SAM

Molecular name is S-adenosylmethionine and molecular weight is 398.44 g/mol.

Drugbank[4] ID: DB00118

SAM is the methyl donor for essentially all known methylation reactions (proteins, DNA, RNA, phospholipids, guanidines). Furthermore, SAM activates the transsulfuration pathway of homocysteine to cysteine, which leads eventually to the production of reduced glutathione (GSH). In addition, SAM provides the propylamine group for the synthesis of polyamines. It is estimated that up to 85% of methylation reactions and 50% of methionine metabolism occurs in the liver. Further mechanisms are described in the methionine sections in Chapter 4.

Plasma levels are typically 60 to 160 nmol/L (24-64 ng/ml) (Struys et al., 2000). SAM crosses the blood-brain barrier (Goren et al., 2004) and is present in cerebrospinal fluid at higher concentrations compared to plasma or serum (Struys et al., 2000).

Data on bioavailability after oral intake of SAM are sparse and has been estimated to be in the magnitude of 1% (Goren et al., 2004; Mischoulon et al., 2012; Yang et al., 2009). The low bioavailability is due to first pass effects, rapid metabolism and chemical instability of the molecule (Goren et al., 2004). Plasma or serum SAM levels increase after oral intake, but to a much lesser extent than after intramuscular or intravenous injection, and oral bioavailability was reported to be within 2-3%. Intake of SAM increased SAM levels slightly, but significantly (Goren et al., 2004). Chronic intake of SAM (1600 mg/day) over six weeks also increased plasma SAM levels, S-adenocylhomocystein levels and, to a lesser extent, homocysteine levels (Mischoulon et al., 2012). SAM did not accumulate with multiple doses (Yang et al., 2009).

SAM has two chiral atoms in the molecule that give different stereoisomers. It is biologically produced from L-methionine and adenosine triphosphate, and can occur as S/S isomers and R/S isomers. The distribution of isomers in pharmaceutical preparation is varying from 45% S/S to 70% S/S isomer (Najm et al., 2004).

There are virtually no data on excretion of SAM.

5.2 Animal studies with SAM

[4] http://www.drugbank.ca.

SAM has been used in numerous animal studies, especially with respect to liver metabolism and the effect of alcohol in non-human primates, baboons, and rats. Most studies report on positive effects of SAM on liver toxicity of ethanol, but not on toxicity of SAM. Only one animal toxicity study with SAM was retrieved from the literature search (after 2002). Ochoa (2009) reported enhancement of the nephrotoxicity of cisplatin (an antitumor drug with known nephrotoxicity) by concurrent application of SAM in mice. This was measured by the increase of plasma creatinine and urea. A possible mechanism is the metabolic activation of cisplatin by SAM in proximal tubule cells. SAM in the absence of cisplatin did neither affect plasma creatinine nor urea (Ochoa et al., 2009).

5.3 Human studies with SAM

In the literature search, five relevant studies in humans and two systematic reviews on the use of SAM as a drug were identified after 2002. SAM has been used as drug applied either intramuscularly, intravenously or orally in osteoarthritis, liver pathologies and depression. In most studies, SAM was used as adjuvant therapy, together with established drugs. Most studies did not state which form of SAM was used and whether stability was controlled. SAM is usually given as enteric coated tablets of 200 mg or 400 mg S-adenosyl-L-methionine disulfate monotosylate salt.

Use of SAM (oral and other routes of administration evaluated together) for the treatment of alcoholic liver pathologies was evaluated and summarised in a Cochrane systematic review (Rambaldi and Gluud, 2006). This review identified six randomised controlled trials with oral administration of SAM in dose 1200 mg SAM per day (two of them were only published as abstract) up to 24 months. The authors conclude that SAM was not associated with serious adverse health effects. No serious adverse health effects were reported in these trials.

Use of SAM for the treatment of osteoarthritis of the hip and the knee was evaluated by a Cochrane systematic review in 2009 (Rutjes et al., 2009). This review identified four trials in which SAM was used orally as the single agent (doses ranging from 600-2400 mg per day). Studies that compared SAM together with other drugs were excluded from the Cochrane review. The RCTs reported on adverse events, and a relative risk of any adverse event was calculated to be 1.27 (95% C.I. 0.94 - 1.71). No information was provided on serious adverse health effects due to SAM intake.

SAM was used in a number of randomised controlled studies in depression, administered either intravenously, intramuscularly or orally. Oral doses usually are between 200 and 1600 mg daily, distributed in 200 mg or 400 mg doses. Studies that used oral SAM reported that depressive symptoms were improved in comparison to placebo, and adverse effects were usually mild and gastrointestinal (constipation, diarrhoea, flatulence) or affected the musculosceletal and nervous system (headache, anxiety, fatigue) (Alpert et al., 2004; Mischoulon and Fava, 2002; Papakostas et al., 2010). However, in patients with bipolar disorders, single cases of mania have been reported (Goren et al., 2004; Mischoulon and Fava, 2002).

5.4 Exposure assessment

SAM is an intermediate in the metabolism of methionine, but it is not occuring naturally in foods. This has been questioned by (Van de Poel et al., 2010), who reported low content of SAM in tomatoes, kiwi and other fruits, in the order of ~ 5 nmol/g fresh weight. However, it can be assumed that dietary intake of SAM from foods is almost absent or very low.

According to the Norwegian Food Safety Authority, there are supplements available on the Norwegian market that contain up to 1200 mg SAM per recommended daily dosage.

5.5 Special groups

There is very little experience with use of SAM in children and adolescents. Adverse effects may have not been reported. Therefore, children and adolescents should only be exposed to SAM under careful evaluation and observation.

SAM acts as an antidepressant and as such improves mood. When used in bipolar disorders, it may induce manic states. Patients with bipolar disorders should be advised not to take SAM unless they are also taking a mood stabiliser (Mischoulon and Fava, 2002).

5.6 Summary SAM

SAM is not, or in very low amounts, occurring in foods but has been widely used as medication in various patient groups (liver disease, osteoarthritis, depression). The oral administration has a low bioavailability, and the products may lack stability over the declared shelf-life. Adverse effects are common, but are mild and affect mostly the gastrointestinal system. Serious adverse events after SAM application have not been reported. The risk for adverse effects was not significantly increased in the studies analysed in the systematic reviews on use of SAM in liver disease (Rambaldi and Gluud, 2006) and osteoarthritis (Rutjes et al., 2009). However, caution may be justified in patients with bipolar disorders as SAM may induce manic states.

The low bioavailability of SAM given orally, and the fact that studies with SAM given intravenously, has not reported on serious adverse events, do not support the setting of an LOAEL for SAM.

5.7 Discussion/conclusion

The low bioavailability of SAM given orally results in a low, but significant, increase in plasma SAM. The adverse effects reported for SAM in the dose range of 200-1600 mg per day (200 mg enteric coated tablets) is primarily gastrointestinal such as constipation, diarrhoea and flatulence, and may be caused by the low bioavailability. Serious adverse advents have not been reported with use of SAM, and a tentative LOAEL could not be given. The induction of manic states in patients with bipolar disorders may be of clinical relevance. Therefore, this patient group should be advised not to take SAM supplements.

Stability and purity may be an issue and should be controlled for.

6 Tryptophan

6.1 Identification and characterisation of tryptophan

Molecular name is L-tryptophan and molecular weight is 204.229 g/mol.

Drugbank[5] ID: DB00150

L-tryptophan is an indispensable (essential) amino acid and serves as a precursor for several small molecules of functional significance, including the vitamin niacin via kynurenine- and quinolin acid, the neurotransmitter serotonin, the metabolite tryptamine, and the pineal hormone melatonin (IOM, 2005). Increased levels of circulating tryptophan have been shown to increase the synthesis of certain neurotransmitters in brain, blood, and other body organs (Fregly et al., 1989; Leathwood and Fernstrom, 1990; Young et al., 1985). Administration of the free form of L-tryptophan prevents pellagra (Hegyi et al., 2004).

Primary product of tryptophan in the liver is kynurenine which is formed when tryptophan is metabolised by tryptophan dioxygenase (Opitz et al., 2011).

The kynurenine pathway of tryptophan metabolism accounts for most of the tryptophan that is not committed to protein synthesis and includes metabolites that are active in the nervous and immune systems (Stone et al., 2012).

The metabolism of L-tryptophan in women given 3.3 mmol L-tryptophan per day and no niacin for seven days is illustrated in Figure 1. The 24-hour urine samples were collected daily for seven days and analysed for each of the L-tryptophan catabolites. Values are seven days means of 10 women; values in parentheses are the percentages of the L-tryptophan consumed (3.3 mmol/day). The figure was prepared using data from (Shibata and Fukuwatari, 2012) with permission.

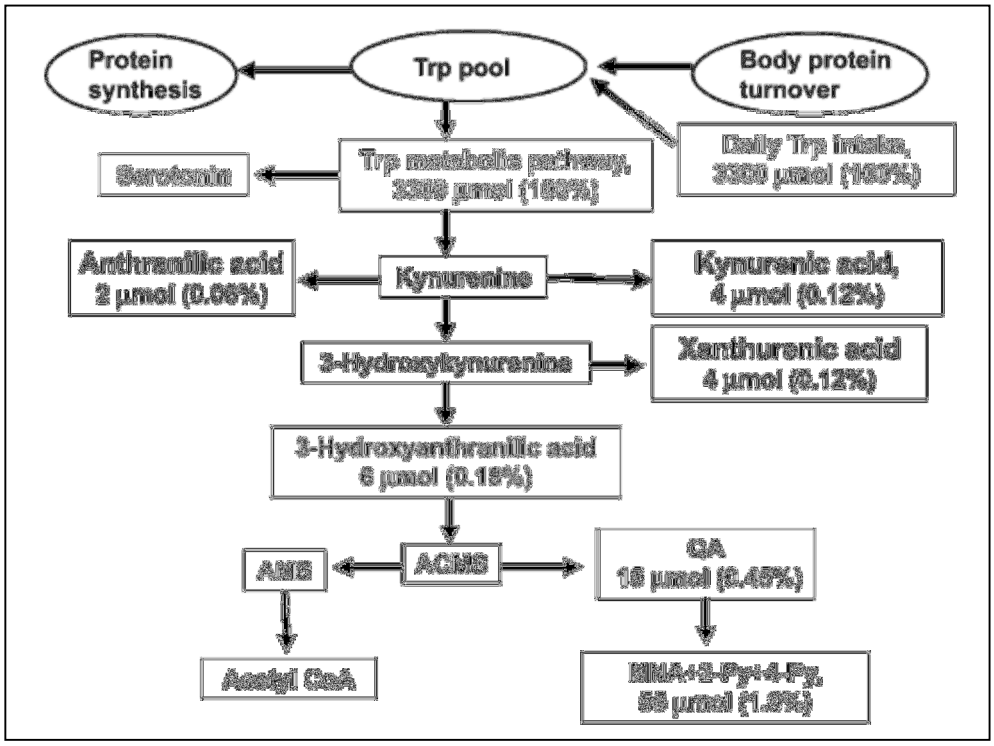

Figure 1: Overview of L-tryptophan metabolism in women. The figure was prepared using data from Shibata and Fukuwatari 2012 with permission.
ACMS: a-amino-b-carboxymuconate-e-semialdehyde.
AMS: a-aminomuconate-e-semialdehyde.

[5] http://www.drugbank.ca.

MNA: N1-methylnicotinamide.
2-Py: N1-methyl-2-pyridone-5-carboxamide.
4-Py: N1-methyl-4- pyridone-3-carboxamide.
QA: quinolinic acid.

6.2 Animal studies with tryptophan

No new animal toxicity studies with L-tryptophan reporting health effects identified in the literature search (after 2002).

6.3 Human studies with tryptophan

In the literature search, five studies in humans were identified after 2002. One study was a study with tryptophan loading among 15 men, age 21-56 years (Forrest et al., 2004), three reports were case reports (Allen et al., 2011; Grangeia et al., 2007; Noakes et al., 2006), one study was an epidemiological study from Japan (Okada et al., 2009).

In the tryptophan loading study among the 15 men 6 g of tryptophan was given once and blood concentration of tryptophan and its metabolites were measured after five and seven hours (Forrest et al., 2004). The men were healthy and between 21 and 56 years of age. The concentration of tryptophan was raised almost five folds after five hours and was still significantly increased 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 was significantly increased after five and seven hours, while kynurenic acid, 3-hydroxy-kynurenine and xanthurenic acid leveled 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 epidemiological study from Japan, 94 subjects were investigated with respect to use of tryptophan supplements and the development of tryptophan associated eosinophilia-myalgia syndrome (EMS) (Okada et al., 2009). The subjects were typed for alleles in 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, OR (95% C.I.) 1.4 (1.1, 1.8), 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.

Three case reports were found, one was an experimental study with a tryptophan metabolite and two were reports of eosinophilia in patients using tryptophan supplementation. In the experimental report the investigator injected himself with a total of 1200 mg of quinolinic acid over a period of one month while monitoring peripheral blood eosinophil counts (Noakes et al., 2006). The eosinophilia count rose from $0.3x10^9$ to $0.8x10^9$ and fell again toward normal count after five weeks. Some infiltration of eosinophils and neutrophils were found in reticular dermis and septa of the paniculus. In the two case reports with patients one women was found with eosinophilia in the lungs (Grangeia et al., 2007) and one in lungs and muscles (Allen et al., 2011) after short periods of supplementation with tryptophan. Although both

women had used tryptophan supplementation in combination with other substances, tryptophan was the common substance and indicated as the causal substance in both reports.

In 2003-2004, the Committee on Toxicity in (COT) UK Food Standard Agency decided, based on the available data, that intake of tryptophan most likely is not associated with the development of EMS. Despite this, a potential association cannot completely be ruled out. COT found that a NOAEL value in humans is 2228 mg tryptophan, as this dose is used therapeutically on a daily basis, and gives no adverse health effects. With the use of an uncertainty factor of 10 they suggested that a daily dosage of 220 mg tryptophan would not present any appreciable risk to health[6].

6.4 Exposure assessment

Daily nutritional requirement is 5 mg/kg body weight. Average intake in all age groups from foods and supplements is 0.9 g/day. Men 51 through 70 years of age had the highest intakes at the 99th percentile of 2.1 g/day (IOM, 2005).

Tryptophan is found in chocolate, dates, milk, yogurt, cottage cheese, read meet egg, fish, meat from poultry, sesame seeds, pies, sun flower seeds, spirulina, beans, peanuts.

According to the Norwegian Food Safety Authority, there are no supplements available at the Norwegian market containing free tryptophan.

6.5 Special groups

Intake of 70–200 mg/kg body weight tryptophan may cause tremor, nausea, and dizziness, and may occur when tryptophan is taken alone or with a drug that enhances serotonin function (e.g., antidepressants, see below) (Glassman and Platman, 1969). The importance of tryptophan supplementation combined with anti-depressant drugs has been studied and shown to be associated with the “serotonin syndrome”. Symptoms of the serotonin syndrome include delirium, myoclonus, hyperthermia, and coma (Boyer and Shannon, 2005). This result indicates that tryptophan supplementation should not be given to patients on anti-depressant drugs.

6.6 Summary tryptophan

Tryptophan is an essential amino acid which is the precursor to niacin, serotonin, tryptamine and melatonin. In the IOM report it is referred that acute and sub-acute use of L-tryptophan is associated with appetite suppression, nausea and drowsiness. However, it is concluded that it was insufficient data to establish a UL for tryptophan. Only one relevant toxicity study in humans with tryptophan was identified after 2002. Intake of 6g tryptophan in 15 healthy men resulted in a significant increase in lipid peroxidation products, indicating an increased stress level. Since no new dose response studies were found, a UL cannot be established. Furthermore, there is still an uncertainty of the role of tryptophan in the development of eosinophilia.

In 2003-2004, the Committee on Toxicity in UK Food Standard Agency considered that available data on tryptophan and EMS showed that tryptophan most likely was not related, but it could not completely be ruled out. On this basis they found that a therapeutic daily

dosage of 2.228 g of tryptophan gave no adverse health effects in humans and used this as a NOAEL value.

It has been shown that the use of L-tryptophan supplementation in combination with anti-depressant drugs has been involved in the development of serotonin syndrome. It is suggested that use of tryptophan supplementation should always be discussed with the physician in patients on anti-depressant drugs.

6.7 Discussion/conclusion

There are only four studies after 2002 which describe adverse effects of tryptophan supplementation. One study indicates increased lipid peroxidation with one single load of tryptophan (6 g). The main concern with L-tryptophan supplementation is nausea, appetite suppression and drowsiness, which is of no toxicological concern, but the involvement in the development of eosinophilia might still be of toxicological concern. Four studies keep the question about the involvement of tryptophan in the development of eosinophilia alive.

The use of 2.228 g of tryptophan as a NOAEL fits well with the finding in the epidemiology study where the impact of tryptophan on eosinophilia showed that the affected group had an intake of 4 g/day of L-tryptophan, whereas the unaffected group used 2.4 g/day. With the use of an uncertainty factor of 10 for between people variations the Committee on Toxicity in UK Food Standard Agency suggested that a daily dosage of 220 mg tryptophan would not present any appreciable risk to health. Although 220 mg tryptophan was considered safe, the UK Committee on Toxicity did not suggest this value as a GL. Nevertheless, it seems reasonable to suggest 220 mg tryptophan as a tentative daily GL.

Individuals taking anti-depressant drugs are advised not to take L-tryptophan supplementation.

7 Purity

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 the L-amino acids of histidine, methionine and tryptophan.

SAM has two chiral atoms in the molecule that give different stereoisomers. It is biologically produced from L-methionine and adenosine triphosphate, and can occur as S/S isomers and R/S isomers, indicating the chirality of the molecule. The first letter refers to the confirmation at the sulphur atom and the second letter to the confirmation at the alpha carbon atom. The distribution of isomers in pharmaceutical preparation is varying from 45% S/S to 70% S/S isomer (Najm et al., 2004).

The biological activity of the R/S isomer is unclear, but the R/S isomer has also been claimed to inhibit methyltransferase reactions.

SAM is an unstable molecule. An investigation from the Food and Drug Administration (FDA) showed that SAM tablets from various lots with a declared content of 200 mg contained between 0 and 220 mg SAM (mean 159 mg, 80%). The proportion of the S-Diasteromer was between 0 and 82%, with a mean of 59% (Hanna, 2004).

Most studies are neither reporting on the form of SAM that have been used, nor on the distribution of isomers. This information is needed.

Impurities in tryptophan supplements from the production process may have been the cause of EMS, and not tryptophan itself. Purity criteria must be complied with in food supplements and foods or drinks fortified with amino acids, and only the L-tryptophan form should be allowed. Purity should be checked by an accredited laboratory.

8 Data gaps

To be able to set a UL, dose-response studies in animals and humans are imperative. It is of great concern that products containing single amino acids with metabolic relevance are allowed on the marked without thorough knowledge of potential toxicity. Although some studies were found where the function of the amino acids was studied, mostly in patient groups, few reported on adverse health effects, and none of these were long term studies. More dose-response studies are needed, including both animal and human studies focusing on possible negative health effects from supplementation with, histidine, methionine, SAM and tryptophan. Long term studies are also necessary to re-evaluate the tentative GLs.

While even high intake of amino acids from dietary proteins seems to be of no physiological concern, the use of single amino acids added to food or as supplements might cause imbalances in the amino acid pool of the body. Very little is known about a possible effect on protein synthesis.

Most studies using SAM have not reported on the stereoisomer distribution of the preparation used (R and S configuration). Any adverse effects could be due to accumulation of the R-stereoisomers or biological effects of these isomers. Reports could be improved by providing the exact chemical nature of the used SAM supplement.

In this risk assessment of the amino acids histidine, methionine, SAM and tryptophan many questions are still left unanswered because of scanty scientific literature.

9 Answer to the terms of reference

The Norwegian Food Safety Authority has requested VKM to conduct a risk assessment of the amino acids histidine, methionine, S-adenosylmethionine and tryptophan added to foods and drinks and in food supplements, and to answer the following questions:

What are the negative health effects of these four amino acids?

Mild adverse effects reported were nausea and reduced appetite with use of all four amino acids. There are indications that intake of methionine during the so called acute methionine-loading test is associated with adverse health effects such as dizziness, nausea, sleepiness and decreased or increased blood pressure. Intake of methionine supplement seems to be a concern for health because increased concentration of its metabolite homocysteine in plasma may be associated with cardiovascular disease.

Intake of tryptophan supplement has resulted in a significant increase in lipid peroxidation products in one study, indicating an increased oxidative stress level. Intake of tryptophan supplement 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.

Are there any special population groups that should avoid one or more of these amino acids?

Persons with hyperhomocysteinemia should avoid methionine supplementation, and patients using anti-depressant medication should avoid tryptophan supplements.

The induction of manic states in patients with bipolar disorders with use of SAM might be of clinical relevance. Therefore, this patient group should be advised not to take SAM supplements.

Can an upper tolerable intake level be set for these amino acids?

Because of no dose-response studies or adverse health effects related to dose were found, UL for these four amino acids could not be established. However, in this assessment a tentative GL at 210 mg is suggested for methionine, and 220 mg is suggested as a tentative daily GL for tryptophan.

What is the population intake of these four amino acids added to foods and drinks and in food supplements?

The intake of these four amino acids in free form was estimated from food supplements sold on the Norwegian market. Intake of free form histidine, SAM and tryptophan seem to be within an acceptable level, whereas methionine supplements available at the Norwegian market is higher than the tentative suggested GL in this assessment.

Annex 1 Search string for the main literature search

1 tryptophan*.ti. (27574)[6]

2 diet supplement*.ti,ab,sh. (63428)

3 nutritional supplement*.ti,ab,sh. (7477)

4 dietary supplement*.ti,ab,sh. (42615)

5 food supplement*.ti,ab,sh. (4042)

6 diet therap*.ti,ab,sh. (53989)

7 adverse effect*.ti,ab,sh. (200715)

8 drug side effect*.ti,ab,sh. (2327)

9 adverse drug reaction*.ti,ab,sh. (129479)

10 drug toxicity.ti,ab,sh. (44203)

11 toxicity.ti,ab,sh. (520206)

12 negative health effect*.ti,ab,sh. (732)

13 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 (981815)

14 1 and 13 (608)

15 limit 14 to yr="2002 -Current" (229)

16 remove duplicates from 15 (140)

[6]Or methionine or histidine.

Annex 2 Tables with included studies

Table 1: Included studies with methionine

Reference	Study design	Exposure	Outcome measures	No. of subjects	Results
Toue et al., 2006	Animal study	0.3, 06, 1.2 or 2.4% methionine by weight of diet	Clinical chemistry, hematology and aminothiols	9 rats/group	2.4% methionine by weight of diet caused a significant increase in the concentration of plasma homocysteine
Krupkova-Meixnerova, 2002	Methionine loading-test	100 mg/kg body weight	Severe complications, mortality rate assessment after 30 days	296 patients with CVD history and 591 controls	No significant increase in mortality rate
O´Dochartaigh et al., 2004	Methionine loading-test	100 mg/kg body weight	Pulmonary function	10 healthy men during exercise	A significant change in respiratory gas exchange process
Cottington et al., 2002	Case-report	May be an overdose (80 g methionine)	Homocysteine	1 woman	A significant increase in plasma homocysteine and methionine concentration. Death after 30 days of methionine loading
Harvey et al., 2003	A multiple case report	Increased methionine intake (up to 8800 mg/L) due to consumption of infant formulas	Methionine and homocysteine	10 babies/children	The concentration of plasma methionine and homocysteine decreased after the withdrawal of infant formulas

Annex 2 Tables with included studies

Table 3: Included studies with SAM

Reference	Study design	Exposure	Outcome measures	No. of subjects	Results
Papakostas et al., 2010	RCT	2 weeks 2x400 mg SAM or placebo followed by 4 weeks 4x400 mg SAM or placebo daily, adjuvant to the SRI 55 patients completed the trial (24 on placebo and 31 on SAM)	Difference in response rate according to HAM-D rating Lipid metabolism, blood pressure Adverse effects	N=73 randomised, N=55 completed the study	Intention to treat analysis Patients on SAM had higher response rates and higher remission rates. No difference in lipid metabolism, higher supine systolic blood pressure, no difference in other blood pressure measurements Adverse effects: 25 in placebo group, 37 in SAM group (not significant)
Mischoulon et al., 2012 (same study to Papakostas et al. 2010)	RCT	2 weeks 2x400 mg SAM or placebo, followed by 4 weeks 4x400 mg SAM or placebo daily, adjuvant to the SRI 55 patients completed the trial (24 on placebo and 31 on SAM)	SAM, SAH, Hcy, Met and 5-MTHF levels before treatment and after treatment	Data in this evaluation are available for 15 patients on placebo and 20 patients on SAM	SAM group: SAM 82.9±22.4 → 504±536 nmol/l SAH 35.7±6.7 → 56.1±18.3 nmol/l Hcy 9.8±2.3 → 10.6±3.6 µmol/l No significant changes in placebo group
Goren et al., 2004	Unblinded trial	1600 mg SAM per day for 4 weeks, no control group	Plasma levels of SAM, methanol, formaldehyde IgG and IgM, formic acid, homocysteine Adverse effects	N=15	SAM 750±120 → 780±120 nmol/l No other significant changes Adverse effects
Yang et al., 2009	Pharmacokinetic trial	1000 mg/day SAM tosylate disulfate orally in 10 subjects for 5 days or 1000 mg/day SAM tosylate disulfate iv for 5 days	Plasma levels of SAM (Cmax, Tmax, AUC0-24, t1/2 after single dose and after 5 days	N=10 oral N=10 iv	Oral SAM, multiple doses Cmax 2.561±1.63 µmol/L Tmax 4.2±1.9 hours T1/2β 4.9±3.0 hours AUC0-24 11.2±5.2µmol/l/h Iv SAM, multiple doses Cmax 138±46 µmol/l Tmax 1.3±0.46 hours T1/2β 4.4±1.15 hours AUC0-24 345±109 µmol/l/h

Reference	Study design	Exposure	Outcome measures	No. of subjects	Results
Rambaldi et al., 2006	Systematic review	Use of SAM in alcoholic liver disease	Effect on alcoholic liver disease Adverse effects	9 RCTs with 434 patients	SAM and non-serious adverse effects (all trials, oral and IV) RR 4.92 (95% CI 0.59 to 40.9) No serious adverse effects reported
Rutjes et al., 2009	Systematic review	Use of SAM in osteoarthritis of the knee and the hip (versus placebo)	Effect on measures of osteoarthritis Adverse effects	4 RCTs with 656 patients	SAM and non-serious adverse effects RR 1.27 (95% CI 0.94 to 1.71) No serious adverse effects reported
Alpert et al., 2004	Open, single treatment trial	Use of SAM (1600 mg/day) for 6 weeks as adjunct in patients with major depressive disorder	Measure of depression (HAM-D score) Adverse effects	N= 30 (22 females)	Improvement of HAM-D score (both in intention-to-treat analysis and in per-protocol analysis Gastrointestinal and musculoskeletal and nervous adverse effects

Annex 2 Tables with included studies

Table 3: Included studies with tryptophan

Reference	Study design	Exposure	Outcome measures	No. of subjects	Results
Forrester et al., 2004	Acute high dose loading with TRP	6 g of acute loading with tryptophan	Oxidative stress, lipid peroxidation products (malondialdehyd and 4-hydroxynonenal)	15 healthy adults	Increased lipid peroxidation after 5 and 7 hours. Significant increase in plasma concentration of tryptophan, kynurenine, kynurenic acid, 3-hydroxykynurenine and 3-hydroxyxyanthranilic acid and quinolinic acid after 5 and 7 hours
Okada et al., 2009	Epidemiological study	Intake of L-tryptophan – gene-environment interaction study	EMS subjects n=28, Ems spectrum disorder n=57 and those unaffected=37 and these were all compared to 872 healthy white controls not using L-tryptophan supplementation.	94 users and 872 non-users	Higher dose of LT (4160 mg/) had an OR of 1.35 (95% C.I. 1.05 – 1.79) compared to the unaffected users (2898 mg/day) and an OR of 1.43 (1.02-2.22) of those not using LT. Age and alleles were also important for EMS development, while sex had no effect
Noakes et al., 2006	1 person was subcutaneous injected with quinolinic acid	Subcutaneous injection of quinolinic acid	The eosinophil count rose from $30x10^{7}$/l to $80x10^{7}$/l. Five weeks after stopping injections the eosinophil count declined to $40x10^{7}$/l.	1	The results indicate that the tryptophan quinolinic acid might be responsible for eosinophilic fasciitis
Grangeia et al., 2007	Case report	Tryptophan (2g/d) 400mg/d of 5-hydroxytryptophan , L-taurine, inositol, panthothenic acid, B_{12}, B_6, L-phosphoserine, phosphothreonine, fluoxetine, carisoprodol and omeprazole	Acute respiratory failure	1	She presented respiratory failure as a manifestation of eosinophilia-myalgia
Allen et al., 2011	Case-report	1500 mg of L-tryptophan for 3 weeks	Blood count, biopsy	1	The women were diagnosed with EMS. In spite of treatment with cortisone the symptoms did not resolve in spite of reduced eosinophilia.

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