



VKM Report 2016:07

# Risk assessment of "other substances" – L-arginine and arginine alpha- ketoglutarate

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

Report from the Norwegian Scientific Committee for Food Safety (VKM) 2016: 07  
Risk assessment of "other substances" – arginine and arginine alpha-ketoglutarate

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Norwegian Scientific Committee for Food Safety  
01.03.2016

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Cover photo: iStock Photo

Suggested citation: VKM. (2016). Risk assessment of "other substances" – L-arginine and arginine alpha-ketoglutarate. Opinion of the Panel on Nutrition, dietetic products, Novel Food an Allergy of the Norwegian Scientific Committee for Food Safety, ISBN: 978-82-8259-197-3, Oslo, Norway.

# **Risk assessment of "other substances" - L-arginine and arginine alpha-ketoglutarate**

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

## **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. Grethe S Tell is acknowledged for her valuable work on this opinion. Jan Alexander (the Scientific Steering Committee), Åshild Krogdahl (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. The risk assessments are the scientific basis for NFSA in its efforts to regulate the use of "other substances".

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

The present report is a risk assessment of the amino acid L-arginine and L-arginine alpha-ketoglutarate (AAKG), a salt of arginine. It is based on published articles retrieved from a literature search and previous risk assessments of L-arginine.

According to information from NFSA, L-arginine is an ingredient in food supplements sold in Norway. NFSA has requested a risk assessment of L-arginine, which according to the information provided by NFSA is found in food supplements in the doses 3000, 3500, 4000, 4500, 5000, 5500, 6000 and 6800 mg/day.

Arginine alpha-ketoglutarate is found in food supplements in doses of 1000 and 2000 mg/day.

Arginine is a constituent of all food proteins. Dairy products, beef, pork, poultry, wild game and seafood, as well as plant sources such as wheat germ and flour, oatmeal and nuts are good sources of arginine.

Arginine is a conditionally essential amino acid, meaning that under most circumstances endogenous synthesis by the human body is sufficient. However, the biosynthetic pathway may under certain conditions produce insufficient amounts. In such cases a dietary supply is needed. Individuals with poor nutrition or certain physical conditions are examples of vulnerable groups.

Under normal conditions, endogenous production of arginine is 15-20 g/day. The requirements for L-arginine in adults are 117 mg/kg body weight (bw) per day (WHO, 2007), i.e. for a 70 kg adult person, the requirement is 8.2 g per day. The mean daily dietary intake for all life stage and gender groups of arginine is approximately 4.2 g/day (1988–1994 NHANES III, USA).

Arginine is physiologically active in the L-form, which is synthesised by endothelial cells and excreted with urine. The major part of body L-arginine is found in proteins. However, L-

arginine is also substrate of nitric-oxide, a potent vasodilator, which may play a major role in regulating blood pressure and improve vascular function.

Arginine, supplied as alpha-ketoglutarate, has been observed to increase nitric-oxide production and is mostly studied in athletes because of its claimed enhancing effect on physical performance.

Due to the lack of adequate scientific information, a no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL) of arginine has not been identified, thus a tolerable upper intake level for arginine has not been established.

Most studies of arginine supplements have been of relatively short duration. The two most relevant randomised placebo-controlled trials for the current risk assessment are those published by Monti et al. (2012) and Lucotti et al. (2009). Both provided a daily dose of 6.4 g arginine, for a duration of 6 and 18 months, respectively. In both studies, adverse events did not differ between arginine and placebo groups.

Thus, based on the studies reviewed as well as previous reports, VKM will use the value 6.4 g/day as value for comparison in the risk characterisation of L-arginine. The dose 6.4 g/day of arginine corresponds to 91 mg/kg bw per day in a 70 kg person.

AAKG is one of several compounds that have been used as a source of arginine in food supplements. It has been studied in healthy athletic men without serious adverse side effects. However, studies of AAKG supplementation are too scarce to draw conclusions for this specific arginine compound.

No data are available indicating whether children or adolescents have different tolerance levels than adults for L-arginine. No tolerance level is set for L-arginine specifically for children or adolescents. The conclusions are therefore based on the assumption of similar tolerance for children and adolescents, per kg body weight, as for adults.

VKM concludes that:

- In adults ( $\geq 18$  years), the specified doses of 3000, 3500, 4000, 4500, 5000, 5500 and 6000 mg/day of L-arginine in food supplement are considered unlikely to cause adverse health effects. The dose 6800 mg/day may represent a risk of adverse health effects.
- In adolescents (14 to  $< 18$  years), the specified doses 3000, 3500, 4000, 4500, 5000, 5500 mg/day L-arginine in food are considered unlikely to cause adverse health effects, whereas the doses 6000 and 6800 mg/day may represent a risk of adverse health effects.
- In children (10 to  $< 14$  years), the specified doses 3000 and 3500 mg/day L-arginine in food supplements are considered unlikely to cause adverse health effects, whereas the doses 4000, 4500, 5000, 5500, 6000 and 6800 mg/day may represent a risk of adverse health effects.

Children below 10 years were not included in the terms of reference.

No dosage of arginine alpha-ketoglutarate in food supplements can be evaluated, due to lack of data.

In terms of the arginine content of AAKG, a dose of 1000 mg AAKG contains 544 mg arginine and 450 mg alpha-ketoglutarate (based on the molecular weight of 174.2 g/mol for arginine and 144.1 g/mol for alpha-ketoglutarate). A dose of 2000 mg AAKG, the highest dose found in food supplements sold in Norway, contains 1088 mg arginine and 900 mg alpha-ketoglutarate. This amount of arginine is well below the lowest specified dose of 3000 mg/day L-arginine found in food supplements.

### **Short summary**

The Norwegian Scientific Committee for Food Safety (VKM) has, at the request of the Norwegian Food Safety Authority, assessed the risk of specified doses of L-arginine and arginine alpha-ketoglutarate in food supplements. VKM concludes that:

- In adults ( $\geq 18$  years), the specified doses of 3000, 3500, 4000, 4500, 5000, 5500 and 6000 mg/day of arginine in food supplements are considered unlikely to cause adverse health effects. The dose 6800 mg/day may represent a risk of adverse health effects.
- In adolescents (14 to  $< 18$  years), the specified doses 3000, 3500, 4000, 4500, 5000, 5500 mg/day L-arginine in food supplements are considered unlikely to cause adverse health effects, whereas the doses 6000 and 6800 mg/day may represent a risk of adverse health effects.
- In children (10 to  $< 14$  years), the specified doses 3000 and 3500 mg/day L-arginine in food supplements are considered unlikely to cause adverse health effects, whereas the doses 4000, 4500, 5000, 5500, 6000 and 6800 mg/day may represent a risk of adverse health effect.

No dosage of arginine alpha-ketoglutarate in food supplements can be evaluated, due to lack of data.

**Key words:** Adverse health effect, negative health effect, Norwegian Scientific Committee for Food Safety, Norwegian Food Safety Authority, other substances, risk assessment, VKM, L-arginine, arginine alpha-ketoglutarate, food supplement

# Sammendrag på norsk

På oppdrag for Mattilsynet har Vitenskapskomiteen for mattrygghet (VKM) vurdert risiko ved tilsetning av "andre stoffer" i kosttilskudd og energidrikk som selges i Norge. VKM har risikovurdert ulike bruksdoser oppgitt fra Mattilsynet. Risikovurderingene gir et vitenskapelig grunnlag for Mattilsynet i arbeidet med å regulere bruken av "andre stoffer".

"Andre stoffer" er stoffer som har en ernæringsmessig eller fysiologisk effekt, og som ikke er vitaminer og mineraler. De tilsettes i hovedsak til kosttilskudd, men også til energidrikker og andre næringsmidler. I disse risikovurderingene har VKM ikke sett på påståtte gunstige helseeffekter, men kun vurdert mulige negative helseeffekter.

I denne rapporten har VKM vurdert risiko ved aminosyren L-arginin og arginin alfa-ketoglutarat (AAKG) (et salt av arginin). Risikovurderingen er basert på tidligere risikovurderinger og publiserte artikler av L-arginin, og publiserte artikler av arginin alfa-ketoglutarat, identifisert i litteratursøk.

Ifølge informasjon fra Mattilsynet er L-arginin en ingrediens i kosttilskudd som selges i Norge. Oppdraget fra Mattilsynet var å risikovurdere følgende doser av L-arginin i kosttilskudd: 3000, 3500, 4000, 4500, 5000, 5500, 6000 and 6800 mg/dag, samt arginin alfa-ketoglutarat i doser på 1000 og 2000 mg/dag.

Arginin finnes i alle matvareproteiner. Melkeprodukter og kjøtt, inkludert storfekjøtt, svin, kylling, vilt og sjømat, samt vegetabiliske produkter som hvete, havre og nøtter er gode kilder.

Arginin er en betinget essensiell aminosyre, det vil si at friske mennesker vanligvis kan produsere tilstrekkelig arginin til å dekke behovet. Imidlertid kan den endogene produksjonen ved spesielle omstendigheter ikke være tilstrekkelig. I slike tilfeller må arginin tilføres utenfra. Individuer med dårlig ernæring eller spesielle tilstander representerer slike sårbare grupper.

Under normale tilstander er den endogene produksjonen av L-arginin 15-20 g per dag. Behovet for L-arginin hos voksne er anslått å være 117 mg/kg kroppsvekt per dag (WHO, 2007), tilsvarende 8,2 g per dag for en voksen person på 70 kg. Gjennomsnittlig dagsinntak for alle aldre er anslått til å være ca. 4,2 g/dag (1988-1994 NHANES III, USA).

Den fysiologisk aktive formen er L-arginin, som syntetiseres blant annet av endotelceller og utskilles i urinen *in vivo*. L-arginin er substrat for nitrogenmonoksyd som utvider blodårene (vasodilatator) og som dermed har en funksjon i regulering av blodtrykket.

Arginin alfa-ketoglutarat øker også nitrogenmonoksyd og er for det meste undersøkt blant idrettsutøvere/atleter på grunn av påstått effekt på muskelstyrke og fysisk prestasjon.

På grunn av manglende vitenskapelig informasjon, er det ikke mulig å bestemme en NOAEL (no observed adverse effect level) eller en LOAEL (lowest observed adverse effect level), og dermed heller ikke et tolerabelt øvre inntaksnivå (UL) for arginin.

De fleste studier av L-arginin har vært av relativt kort varighet. De to mest relevante randomiserte placebokontrollerte studiene for denne risikovurderingen er publisert av Monti et al. (2012) og Lucotti et al. (2009). I begge studier brukte man daglige doser på 6,4 g arginin og de varte i henholdsvis 6 og 18 måneder. Hyppigheten av negative helseeffekter var ikke forskjellige i intervensjonsgruppen (arginin) og placebogruppen.

Basert på studiene som er vurdert, samt tidligere rapporter om arginin, vil VKM bruke en "value for comparison" i risikovurderingen av arginin på 6,4 g/dag. Dette tilsvarer 91 mg/kg kroppsvekt/dag hos en person på 70 kg.

Arginin alfa-ketoglutarat er en av flere forbindelser som har blitt brukt som kilde for arginin i kosttilskudd. Arginin alfa-ketoglutarat er studert blant friske atletiske menn uten at alvorlige bivirkninger ble rapportert. Det er imidlertid for få studier av arginin alfa-ketoglutarat til å kunne konkludere angående mulig risiko forbundet med arginin alfa-ketoglutarat som kosttilskudd.

Det er ikke funnet data som antyder at barn eller ungdom har en annen toleranse enn voksne for L-arginin, og det er ikke fastsatt toleransenivå spesielt for barn eller ungdom. Konklusjonene er derfor basert på en forutsetning om at barn og ungdom har samme toleranse for arginin som voksne, per kg kroppsvekt.

Vitenskapskomiteen for mattrygghet (VKM)s konkluderer med at:

- For voksne ( $\geq 18$  år) er det usannsynlig at de spesifiserte dosene 3000, 3500, 4000, 4500, 5000, 5500 eller 6000 mg/dag L-arginin i kosttilskudd vil forårsake negative helseeffekter. En dose på 6800 mg/dag vil kunne representere en risiko for negative helseeffekter.
- For ungdom (14 to  $<18$  år), er det usannsynlig at de spesifiserte dosene av 3000, 3500, 4000, 4500, 5000 og 5500 mg/dag L-arginin i kosttilskudd vil forårsake negative helseeffekter. Dosene på 6000 og 6800 mg/dag vil kunne representere en risiko for negative helseeffekter.
- For barn (10 til  $<14$  år), er det usannsynlig at de spesifiserte dosene 3000 og 3500 mg/dag L-arginin i kosttilskudd vil forårsake negative helseeffekter. Dosene på 4000, 4500, 5500, 6000 og 6800 mg/dag vil kunne representere en risiko for negative helseeffekter.

Barn under 10 år inngår ikke i oppdraget.

På grunn av manglende data, kan ingen doser av arginin alfa-ketoglutarat i kosttilskudd evalueres.

Med hensyn til arginindelen av AAKG, inneholder en dose på 1000 mg AAKG 544 mg arginine og 450 mg alfa-ketoglutarat (basert på molekylvekten 174,2 g/mol for arginine og 144,1 for

alfa-ketoglutarat). En dose på 2000 mg AAKG, den høyeste dosen AAKG solgt i Norge, inneholder 1088 mg arginin, som er under den laveste spesifiserte dosen for arginin på 3000 mg/dag L-arginin i kosttilskudd.

### **Kort sammendrag**

På oppdrag for Mattilsynet har Vitenskapskomiteen for mattrygghet (VKM) vurdert risiko ved inntak av spesifikke doser L-arginin og arginin alfaketoglutarat i kosttilskudd. VKM konkluderer med at:

- For voksne (> 18 år) er det usannsynlig at de spesifiserte dosene 3000, 3500, 4000, 4500, 5000, 5500 eller 6000 mg/dag L-arginin i kosttilskudd vil forårsake negative helseeffekter. En dose på 6800 mg/dag vil kunne representere en risiko for negative helseeffekter.
- For ungdom (14 til <18 år), er det usannsynlig at de spesifiserte dosene av 3000, 3500, 4000, 4500, 5000 og 5500 mg/dag L-arginin i kosttilskudd vil forårsake negative helseeffekter. Dosene på 6000 og 6800 mg/dag vil kunne representere en risiko for negative helseeffekter.
- For barn (10 til <14 år), er det usannsynlig at de spesifiserte dosene 3000 og 3500 mg/dag L-arginin i kosttilskudd vil forårsake negative helseeffekter. Dosene på 4000, 4500, 5500, 6000 og 6800 mg/dag vil kunne representere en risiko for negative helseeffekter.
- På grunn av manglende data, kan ingen doser av arginin alfa-ketoglutarat i kosttilskudd evalueres.

# Abbreviations and glossary

## Abbreviations

AAKG	- arginine alpha-ketoglutarate
ADMA	- asymmetric dimethylarginine
ASL	- argininosuccinate lyase
ASS	- argininosuccinate synthetase
AMP	- adenosine monophosphate
ATP	- adenosine triphosphate
bw	- body weight
DDAH	- dimethylarginine dimethylaminohydrolase
EFSA	- European Food Safety Authority
LOAEL	- lowest observed adverse effect level
NFSA	- Norwegian Food Safety Authority [ <i>Norw.</i> : Mattilsynet]
NHANES III	- Third National Health and Nutrition Examination Survey
NO	- nitric oxide
NOS	- nitric oxide synthase
NOAEL	- no observed adverse effect level
OSL	- observed safe level
RCT	- randomised controlled trial
STEMI	- ST-segment elevation myocardial infarction
UL	- tolerable upper intake level
VKM	- Norwegian Scientific Committee for Food Safety [ <i>Norw.</i> : 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. VKM uses the definition established by World Health Organization (WHO) for "adverse effect": a change in morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences (WHO, 1994).

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

# Background as provided by the Norwegian Food Safety Authority

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

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

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

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. 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-arginine and arginine alpha-ketoglutarate in food supplements at the following doses:

L-arginine: 3000, 3500, 4000, 4500, 5000, 5500, 6000 and 6800 mg/day

Arginine alpha-ketoglutarate: 1000 and 2000 mg/day

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

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

# Assessment

## 1 Introduction

"Other substances" are described in the food supplement directive 2002/46/EC as *substances other than vitamins or minerals that have a nutritional or physiological effect*, and may be added to food supplements or e.g. energy drinks. VKM has in this series of risk assessments of "other substances" not evaluated any potential beneficial effects from these substances, but merely possible adverse effects at specified doses sold in Norway.

This risk assessment regards the substance L-arginine and arginine alpha-ketoglutarate *per se*, and no specific products.

According to information from the Norwegian Food Safety Authority (NSFA), L-arginine is an ingredient in food supplements purchased in Norway. NSFA has requested a risk assessment of the intake of 3000, 3500, 4000, 4500, 5000, 5500, 6000 and 6800 mg L-arginine/day from food supplements, and of 1000 mg and 2000 mg daily intake of arginine alpha-ketoglutarate (a salt of arginine). Estimated from NHANES III, mean arginine intake for the US adult population was 4.2 g/day, while men 51 through 70 years had the highest reported intake at the 99<sup>th</sup> percentile of 10.1 g/day (IOM, 2005).

### **L-arginine**

Arginine is a conditionally essential amino acid, meaning that under most circumstances the body synthesis is adequate, and a dietary supply is not necessary. In adults with an adequate protein intake, endogenous synthesis is sufficient to cover physiological needs. Arginine supply through the diet is essential during growth and may be needed during recovery following injury. Under normal conditions, endogenous daily production of 15-20 g occurs via the citrulline intestinal-renal axis (Barbul and Uliyargoli, 2007).

Arginine is physiologically active in the L-form, which is synthesised by endothelial cells, i.e. in the inner lining of the blood and lymph vessels and is excreted with urine. Most dietary L-arginine is incorporated in proteins in the liver. However, arginine is also the substrate for the enzyme nitric oxide synthase (NOS) which is responsible for the production of nitric oxide (NO), an endogenous messenger molecule and vasodilator involved in many of the processes associated with the development of atherosclerosis, including the regulation of blood pressure. Several studies have therefore been conducted to examine whether dietary L-arginine supplementation can augment NO production in humans and thereby improve vascular health. L-arginine also has an essential metabolic role in the formation of urea and creatine. Moreover, it is important in the release of hormones (Guest et al., 2004). Consequently, the most important functions of L-arginine, in addition to its role as an essential constituent of all proteins, include: i) increasing the secretion of hormones such as

growth hormone, insulin, glucagon and prolactin (Isidori et al., 1981; McConell, 2007); ii) improving endothelial function (Dioguardi, 2011); iii) improving the immune function (Munder, 2009); iv) increasing NO production (Elam, 1988).

### **Arginine alpha-ketoglutarate (AAKG)**

AAKG is a salt of the amino acid arginine and alpha-ketoglutaric acid. Both components are intermediates in the metabolism of nitric oxide. AAKG is the keto acid produced by deamination of glutamate, and is an intermediate in the Krebs' cycle.

AAKG is mostly studied in athletes because of its claimed enhancing effect on physical performance.

# 2 Hazard identification and characterisation

## 2.1 Literature

The present risk assessment of L-arginine is based on previous risk assessments and articles retrieved from literature search. The risk assessment of AAKG is based on a literature search as there are no previous risk assessments.

### 2.1.1 Previous risk assessments

Risks related to L-arginine supplementation have previously been evaluated by the Institute of Medicine (IOM) in USA in 2005, VKM in 2011, and the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (ASEAN) in 2012.

#### ***Dietary reference intakes, tolerable upper intake levels for individual amino acids. Institute of Medicine (IOM), USA, 2005***

IOM attempted to establish an upper tolerable intake level (UL) for amino acids in 2005, including arginine. Both animal and human studies were reviewed. The animal studies included are described in section 2.4.2. However, none of the studies, human or animal, could be used to establish a NOAEL or a LOAEL (IOM, 2005).

The IOM (2005) summarised their review as follows:

"Studies of oral administration of supplemental arginine in humans (in excess of normal dietary intakes of approximately 5.4 g/100 g of mixed dietary proteins) were not designed to systematically study the toxicity of chronic oral exposure to this amino acid. They are generally of short duration, do not present dose-response data, and involve small numbers of individuals. Although data from these studies do not support the development of a LOAEL and thus a UL, they do give some indication of the effects from oral arginine intakes of up to 30 g/day. Oral intakes of arginine aspartate providing 5 and 10 g/day of free arginine for 80 days resulted in dose-related weight increases, digestive disturbances, and sleepiness (De Aloysio et al., 1982). Daily intakes of 20 to 30 g of arginine hydrochloride for 7 to 14 days resulted in gastrointestinal disturbances (Barbul et al., 1990; Barbul et al., 1981). Such effects were considered mild and responded to lowering the oral dose at various times during the day without affecting the total daily intake."

The IOM concluded that "Although the data appear to indicate minimal effects from arginine supplementation at intakes up to 24.8 g/day of free arginine base, the unconfirmed finding that 30 g/day of arginine for 3 days resulted in a stimulation of tumor growth in breast

cancer patients (Park et al., 1992) indicates that dietary supplementation with arginine is not advisable other than in at-risk children with congenital defects of argininosuccinic acid synthetase or argininosuccinase. Therefore, since neither a no observed adverse effect level (NOAEL) nor lowest observed adverse effect level (LOAEL) can be identified for intake of L-arginine from dietary supplements in healthy individuals, a UL (tolerable upper level) could not be determined".

Animal studies cited in the IOM (2005) are briefly discussed in chapter 2.4.2.

### ***VKM report on risk categorisation of amino acids. Norway, 2011***

In 2011 VKM conducted a risk categorisation of about 30 amino acids and amino acid compounds according to potential health risks related to high intakes of the amino acids (VKM, 2011). This categorisation was based on a comprehensive MEDLINE literature search including both human and animal studies. No animal studies were found relevant for inclusion, and a few human studies were identified, mostly the same as those included in the IOM report mentioned above. No human studies were identified which had a special focus on potential negative health effects from oral supplementation with L-arginine. The human studies that had been conducted had been of short duration and included few individuals. The report cited the review by Shao and Hathcock (2008) (chapter 2.4.1.3) who had suggested that since a NOAEL or LOAEL cannot be identified, the term "observed safe level" (OSL) should be used and this should be 20 g/day.

L-arginine was categorised as an amino acid with moderate risk. It was emphasised that the VKM report from 2011 has several limitations and can only be regarded as a preliminary report and not as a risk assessment of amino acids.

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

The Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) report published in 2012 included a review of studies conducted in humans, and concluded that "In the revision of 38 clinical tests on humans, no adverse effects or clinical alterations were found, not therefore permitting the establishment of a value for the NOAEL or LOAEL for the oral administration of L-arginine (AESAN, 2012). The Scientific Committee concluded that, based on the information available to date and taking into account the general considerations reflected in this report, the AESAN proposal of a maximum daily amount of 3 g of L-arginine is acceptable from the safety point of view for use as a food supplement." The rationale for selecting 3 g/day was not provided.

The ASEAN report concluded that since neither a NOAEL nor LOAEL could be identified for intake of L-arginine from dietary supplements in healthy individuals, a UL could not be determined.

## **2.1.2 Literature search**

### ***2.1.2.1 Search strategy***

Literature searches were performed in MEDLINE and EMBASE in order to retrieve publications on adverse effects caused by L-arginine or AAKG, without any restrictions regarding year of publication. These databases were chosen to ensure comprehensive study retrieval. The main literature search for human studies with L-arginine was performed by a librarian 27 March and updated 6 October 2015. An additional search for AAKG was conducted 10 September 2015, the latter also including animal studies. Finally, a search for animal studies with arginine was conducted 10 November 2015. The strategies for the searches are included in Appendix 1.

### ***2.1.2.2 Publication selection***

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

- L-arginine or AAKG in relation to adverse effect must be addressed in the title, abstract or full text of the paper
- Result not affected by other substances than L-arginine or AAKG
- Oral route of exposure to L-arginine or AAKG
- Human studies were performed in apparently healthy individuals or patient groups who are assumed to have normal L-arginine or AAKG absorption and metabolism

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

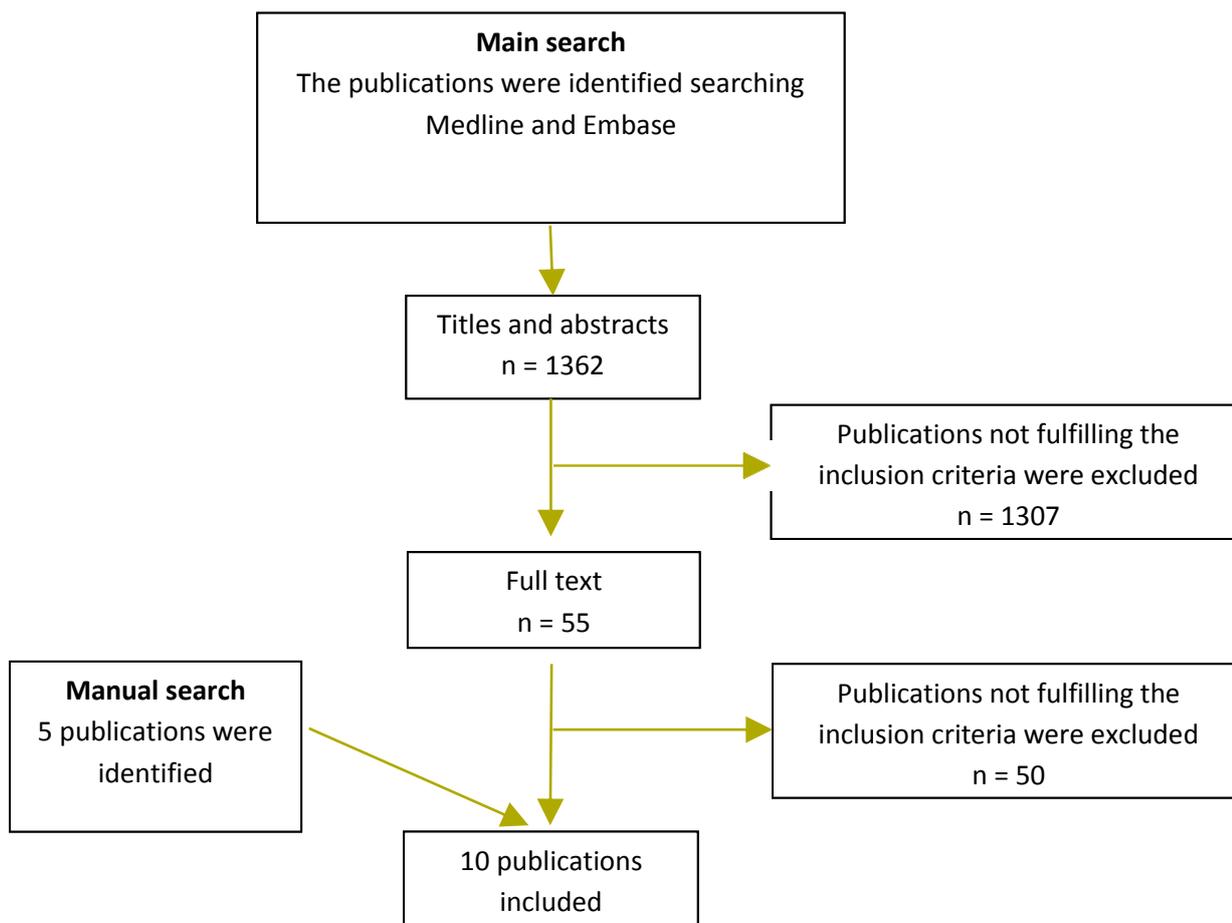
The literature search 27 March 2015 identified 1362 articles.

Study titles and abstracts were first reviewed by the secretariat, followed by a further selection by the author, resulting in selection of 55 full text articles. After review of the available full text articles, 5 articles were included. The updated search on 6 October 2015 did not yield any additional papers fulfilling the inclusion criteria.

Additionally, 5 studies from manual searching/retrieval of relevant literature cited in the full-text papers have been identified and are included.

Two studies from the literature search for AAKG were included. No animal studies were found relevant for risk assessment of arginine.

A final total of 10 publications were identified and included in the results in this report (see Figure 2.1.2.2-1).

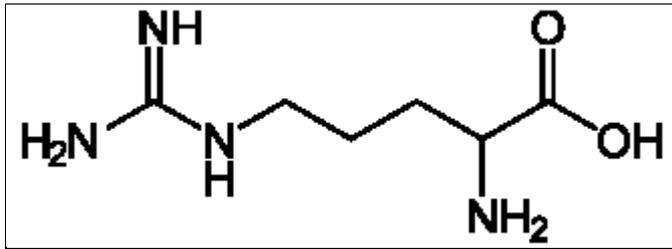


**Figure 2.1.2.2-1:** Flowchart for the literature search for L-arginine and the subsequent publication selection.

## 2.2 General information

### 2.2.1 Chemistry

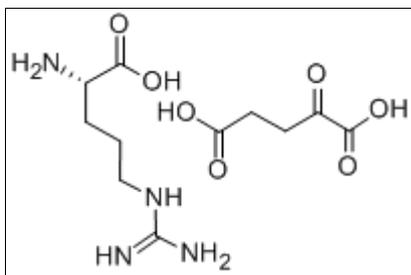
L-arginine,  $C_6H_{14}N_4O_2$  (2-amino-5-guanidinopentanoic acid), is a conditionally essential  $\alpha$ -amino acid. The CAS number is 74-79-3, and molweight is 174.20 g/mol. Figure 2.2.1-1 shows the structural formula for L-arginine.



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

Alpha-ketoglutarate is a five-carbon dicarboxylic acid produced in the citric acid cycle from the oxidative decarboxylation of isocitrate. In a subsequent decarboxylation reaction, AKG is converted to succinyl coenzyme A, a reaction catalyzed by the AKG dehydrogenase complex.

L-arginine alpha-ketoglutarat,  $C_6H_{14}N_4O_2 \cdot C_5H_6O_5$ , has the CAS number 16856-1, and molweight is 320.30 g/mol. Figure 2.2.1-2 shows the structural formula for L-arginine alpha-ketoglutarat.



**Figure 2.2.1-2:** Structural formula of L-arginine alpha-ketoglutarate (AAKG).

## 2.2.2 Occurrence

L-arginine is found in all food proteins. Red meat, poultry, fish and dairy products, and in high quality plant proteins such as soy protein are good dietary sources.

According to the information provided by the Norwegian Food Safety Authority, L-Arginine is found in food supplements in the doses 3000, 3500, 4000, 4500, 5000, 5500, 6000 and 6800 mg/day. AAKG is found in food supplements in doses of 1000 and 2000 mg/day.

Under normal conditions the endogenous daily arginine production 15-20 g. The synthesis occurs via the citrulline intestinal-renal axis (Barbul and Uliyargoli, 2007).

## 2.3 Absorption, distribution and metabolism

### 2.3.1 In humans

#### L-arginine

Dietary arginine is absorbed in the small intestine. A large proportion of arginine is used in protein synthesis, approximately 5% in urea synthesis, and a small portion (<5%) is utilised by the NOS enzyme system for conversion to NO (Chetty, 2010).

Endogenously, arginine is synthesised from citrulline by the sequential action of the cytosolic enzymes argininosuccinate synthetase (ASS) and argininosuccinate lyase (ASL). In terms of energy, this is costly, as the synthesis of each molecule of argininosuccinate requires hydrolysis of adenosine triphosphate (ATP) to adenosine monophosphate (AMP), i.e., two ATP equivalents. In essence, taking an excess of arginine gives more energy by saving ATPs that can be used elsewhere.

Endogenous citrulline can be derived from multiple sources: from arginine via nitric oxide synthase (NOS), from ornithine via catabolism of proline or glutamine/glutamate, or from asymmetric dimethylarginine (ADMA) via dimethylarginine dimethylaminohydrolase (DDAH).

The pathways linking arginine, glutamine and proline are bidirectional. Thus, the net utilisation or production of these amino acids is highly dependent on cell type and developmental stage.

The epithelial cells of the small intestine produce citrulline, primarily from glutamine and glutamate. The proximal tubule cells of the kidney extract citrulline from the circulation and convert it to arginine, which is returned to the circulation. As a consequence, impairment of small bowel or renal function can reduce endogenous arginine synthesis, thereby increasing the dietary requirement.

Synthesis of arginine from citrulline also occurs at a low level in many other cells, and cellular capacity for arginine synthesis can be markedly increased under circumstances that also induce NOS. Thus, citrulline, a coproduct of the NOS-catalysed reaction, can be recycled to arginine in a pathway known as the citrulline-NO or arginine-citrulline pathway. This is demonstrated by the fact that, in many cell types, citrulline can substitute for arginine to some degree in supporting NO synthesis. However, recycling is not quantitative because citrulline accumulates along with nitrate and nitrite, the stable end-products of NO, in NO-producing cells.

Arginine, ornithine, and lysine also share a common uptake and transport system in the brain, leukocytes, erythrocytes, fibroblasts, and leukocytes (Barbul and Uliyargoli, 2007).

## 2.4 Toxicological data/Adverse effects

### 2.4.1 Human studies

Oral supplementation with arginine increases local nitric oxide (NO) production in the small intestine and this may be harmful under certain circumstances (Gimble, 2007). Therefore, many studies have examined gastrointestinal adverse events, including diarrhea, nausea and vomiting, adverse events that are suitable for self-reporting by study participants. L-arginine also has the potential to reduce vascular stiffness, thus several studies have investigated whether arginine supplements improve vascular health, including reducing the risk of pre-eclampsia. No studies have reported adverse effects on vascular stiffness. We are not aware of studies examining a possible risk of syncope. None of the studies reviewed aid in the assessment of establishing a safe upper intake level.

As a dietary supplement, Arginine-HCl is mostly used, however most studies reviewed do not specify which chemical form of arginine that was used. An overview of the included studies investigating arginine or AAKG and adverse health effects is given in Tables 2.4.1-1, 2.4.1-2 and 2.4.1-3.

**Table 2.4.1-1:** Overview of human RCTs investigating L-arginine and adverse health effects.

Reference	Participant characteristics, age groups	Country	Number in treatment group		Doses, g/day	Main endpoints	Length of follow-up	Adverse effects
			Arginine	Control				
Monti et al. (2012)	Patients with impaired glucose tolerance and metabolic syndrome	Italy	72	72	2 x 3.2 g/day or placebo	Prevention or delaying diabetes mellitus type 2	18 months	Adverse effects did not differ between arginine and placebo groups
Lucotti et al. (2009)	Nondiabetic patients with stable coronary artery disease	Italy	16	16	6.4 g/day or placebo	Endothelial function, insulin sensitivity, inflammation	6 months	No patients manifested adverse events
Micker et al. (2007)	Patients with peripheral arterial occlusive disease	Poland	24	24	3x4 g/d or placebo	Pain free-walking distance and monitoring of side effects	28 days	No adverse effects were reported
Wilson et al. (2007)	Subjects with intermittent claudication	USA	66	67	3 x 1 g/day or placebo	Vascular reactivity and functional capacity	6 months	No group differences in serious or total adverse events were found
Lucotti et al. (2006)	Obese type 2 diabetic patients	Italy	16	17	8.3 g/day or placebo	Several biomarkers of glucose and insulin metabolism	21 days	No adverse event was reported in either group
Schulman et al. (2006)	Patients with acute ST-segment elevation myocardial infarction	USA	78	75	3 x 3 g/day or placebo (Arg-HCl)	Vascular stiffness and ejection fraction	6 months (stopped due to deaths in the Arg group)	Significantly increased mortality, 6 patients in the Arg group vs 0 in placebo. The trial was stopped. No significant differences in other adverse events

**Table 2.4.1-2:** Overview of human reviews and other studies investigating L-arginine and adverse health effects.

Reference	Participant characteristics, age groups	Country	Doses, g/day	Main endpoints	Length of follow-up	Adverse effects
<b>Reviews</b>						
Gui et al. (2014)	7 RCTs with 916 women with hypertensive disorders of pregnancy	World-wide	Intravenous or oral, 3 - 30 g/day	Incidence of pre-eclampsia or eclampsia	Less than 9 months	3 studies reported some adverse effects (nausea, dyspepsia, vomiting, dizziness, palpitations and headache) of oral arginine supplementation, while no adverse effects were reported in two of the studies. No teratogenic or lethal effects were reported
Kang et al. (2014)	RCTs of various patient groups with compromised immune systems. 11 trials, 321 patients	World-wide	0.2 – 30 g/day	Immune function (CD4+ T-cell proliferation response)	6 – 30 days	The L-arginine-supplemented group had significantly lower incidence of infections. No differences in length of hospital stay
Shao and Hathcock (2008)	RCTs of healthy adults and various patient groups	World-wide	Up to 42 g/day	Diverse adverse events	At least one week	An observed safe level of 20 g/day is suggested
<b>Other studies</b>						
Evans et al. (2004)	Uncontrolled study of 12 healthy subjects	USA	3, 9, 21 and 30 g/day (free acid)	Serum levels and reported side effects	1 week periods at different doses	9 g/day had minimal side effects. 21 and 30 g/day were associated with some adverse effects, mostly diarrhea

**Table 2.4.1-3:** Overview of human studies investigating arginine alpha-ketoglutarate and adverse health effects.

Reference	Participant characteristics, age groups	Country	Number in treatment group		Doses, mg/day	Main endpoints	Length of follow-up	Adverse effects
			AAKG	Control				
<b>RCTs</b>								
Willoughby et al. (2011)	Physically active men	USA	12	12	12 g/day of NO(2) Platinum (trade name)	Hemodynamics , brachial-artery blood flow and circulating levels of metabolites	7 days	None of the participants reported any adverse events associated with ingesting intervention supplement or placebo
Campbell et al. (2006)	Resistance-trained men	USA	20	15	3 x 4g/day	Bench press and other strength measures	8 weeks	No significant clinical side effects were reported

### 2.4.1.1 Randomised controlled trials (RCT)

#### Effect of a long-term oral L-arginine supplementation on glucose metabolism: a randomized, double-blind, placebo-controlled trial. Monti et al., 2012

This study was designed to assess the safety and efficacy of a supplementation of 6.4 g/day, given as 3.2 grams of L-arginine two times per day, versus placebo (Monti et al., 2012). One hundred and forty four patients with impaired glucose tolerance and metabolic syndrome were randomised to receive 6.4 g L-arginine or placebo daily for 18 months, plus a 12-month

extended follow-up period after study drug termination. The purpose was to examine whether L-arginine prevented or delayed type 2 diabetes mellitus. At each clinic visit, every 6 months, data for adverse events were collected. It is not reported how this collection was conducted or whether prespecified conditions were ascertained. Adverse events were reviewed by the principal investigator who was blinded to assignment.

During the study period one episode of gall bladder stone and one episode of dizziness were reported with L-arginine while one episode of psoriatic arthritis, one episode of angina pectoris and one episode of supraventricular tachycardia was reported with placebo. Adverse events did not differ significantly between arginine and placebo groups.

### **Oral L-arginine supplementation improves endothelial function and ameliorates insulin sensitivity and inflammation in cardiopathic nondiabetic patients after an aortocoronary bypass. Lucotti et al., 2009**

This was a randomised controlled trial of 32 nondiabetic patients who had stable coronary artery disease after a successful coronary artery bypass grafting (Lucotti et al., 2009). The patients received 3.2 g L-arginine two times per day, or placebo, for 6 months. The purpose of the study was to examine whether long-term arginine treatment was beneficial. Endothelial function, insulin sensitivity and inflammation were monitored. Visits were performed every 2 months. The authors reported that: "No gastrointestinal distress and no adverse events were recalled during the whole study period". No patients interrupted the treatment during the study period or manifested adverse events (not defined).

### **The influence of oral supplementation of L-arginine on intermittent claudication in patients with peripheral arterial disease of the lower extremities. Micker et al., 2007**

The purpose of this randomised controlled trial was to estimate the effect of 28-days supplementation of L-arginine on intermittent claudication in patients with peripheral arterial disease (Micker et al., 2007). Forty eight patients were randomised to receive L-arginine 3x4 g/day or placebo. Patients were under 'close clinical supervision' and monitored for drug tolerance and possible side effects (how the monitoring was conducted or side effects defined are not described). During the study one patient experienced dyspeptic symptoms which receded after taking an H<sub>2</sub>-blocker. No other adverse effects (not defined) were reported.

### **L-arginine supplementation in peripheral arterial disease. No benefit and possible harm. Wilson et al., 2007**

A randomised clinical trial of 133 subjects with intermittent claudication was conducted. Patients were randomised to receive 3 g/day of L-arginine or placebo, for 6 months. Outcomes were vascular reactivity and functional capacity, measured as maximum walking distance (absolute claudication distance [ACD]). L-arginine did not increase nitric oxide synthesis or improve vascular reactivity (Wilson et al., 2007). The improvements in ACD

during the trial was significantly lower in the arginine group compared to placebo which the authors suggest could indicate an adverse effect of L-arginine on functional capacity.

A questionnaire was used to obtain information about possible adverse events. Nine serious adverse events were reported in each trial arm, including acute coronary syndrome, cancer and gastrointestinal hemorrhage. Other adverse events were reported by 35 in the placebo group and 46 in the L-arginine group. The number of total adverse events was 44 in the placebo and 55 in the L-arginine group. The authors concluded that no group differences in serious or total adverse events were found.

**Beneficial effects of a long-term oral L-arginine treatment added to a hypocaloric diet and exercise training program in obese, insulin-resistant type 2 diabetic patients. Lucotti et al., 2006**

The aim was to evaluate the effects of a long-term oral L-arginine therapy on several biomarkers of diabetes and the metabolic syndrome (Lucotti et al., 2006). 33 type 2 diabetic patients were randomised to receive 8.3 g/day of L-arginine or placebo for 21 days. Patients were in hospital during the study. No adverse event (not defined) was reported in either group. It is not described how adverse events were ascertained.

**L-arginine therapy in acute myocardial infarction. The vascular interaction with age in myocardial infarction (VINTAGE MI) randomized trial. Schulman et al., 2006**

One hundred and fifty three patients with acute ST-segment elevation myocardial infarction (STEMI) were randomised to receive 1 g L-arginine (as Arg-HCl) three times daily for one week, increasing to 2 g three times daily in week two, followed by 3 g three times daily in week 3 (Schulman et al., 2006). Patients were maintained at this dose for 6 months. Outcomes were vascular stiffness and cardiac ejection fraction. Patients were enrolled from February 2002 through June 2004. Interim analyses for safety and end-points were performed every six months, and the data safety and monitoring committee terminated the study early for safety concerns. Death occurred in 6 patients (8.6%) in the L-arginine group and none in the placebo group (P=0.01). No group differences in the outcome measures were found. No differences in plasma levels of L-arginine were found between the arginine and placebo arms, nor a dose-related difference in L-arginine levels at 6 months. The proportion of adverse events, including gastrointestinal symptoms, flushing and dizziness, were 43% in the arginine group and 36% in the placebo group, these percentages were not significantly different.

The authors discuss several potential mechanisms by which L-arginine therapy may be harmful in post-myocardial infarction patients, including that high levels of NO may contribute to the myocardial dysfunction and systemic vasodilatation that occur in both septic and cardiogenic shock.

Because an increased mortality rate after arginine supplementation, compared to placebo, has not been found in other trials of patients with cardiovascular disease, the enrollment of patients within 3 to 21 days after a first ST-elevation acute myocardial infarction, in a period of an unstable cardiovascular condition, has been put forth as an explanation for the increased mortality rate observed in this study. Other arginine trials of cardiovascular disease patients that have enrolled patients a longer time after their infarction have not observed any increased risk in the arginine group (Lucotti et al., 2009).

#### ***2.4.1.2 Other studies***

##### **Biochemical responses of healthy subjects during dietary supplementation with L-arginine. Evans et al., 2004**

In this uncontrolled experimental study, twelve healthy subjects took daily doses of 3, 9, 21 and 30 g/day of L-arginine (as free amino acid) for one-week periods, starting with the lowest dose (Evans et al., 2004). The mean serum concentration of L-arginine reached a peak at 9 g/day, however, individual responses differed markedly. 9 g/day was associated with "minimal side effects" (not further defined in the article). Ten of 12 subjects reported adverse effects a doses of 21 g/day. Of these, 4 reported diarrhea, 1 trembling, 1 vomiting and 1 reported nose bleeding. At 30 g/day, 9 of 10 reported diarrhea, 1 of headache and 1 of dry mouth. The authors were surprised at the relatively large proportion of subjects who reported side effects. They discussed that this may be due to the biochemical form of the supplement used; as free acid rather than L-arginine-HCl.

#### ***2.4.1.3 Reviews and metaanalyses***

##### **Arginine supplementation for improving maternal and neonatal outcomes in hypertensive disorders of pregnancy: A systematic review. Gui et al., 2014**

Four randomised controlled trials of oral arginine supplementation to women with hypertensive disorders of pregnancy were included (Gui et al., 2014). A total of 643 women were included in these trials, and studies used different doses. One study of 80 patients used 4 g/day arginine versus placebo for 2 weeks, here 3 of the 39 patients receiving L-arginine reported diarrhea. The studies using either 3 g/day for 3 weeks, or 12 g/day for 2 week did not report any adverse effects.

##### **Effect of L-arginine on immune function: a meta-analysis. Kang et al., 2014**

This meta-analysis/systematic review was performed to assess whether L-arginine supplementation could improve the specific markers of immune function, and to evaluate the safety of L-arginine supplementation with regard to infections (Kang et al., 2014). Randomised controlled trials published in the period 1996-2013 were searched for. Data from 11 trials involving 321 patients were included, doses ranged from 0.2 g/day for 6 days to 30 g/day for 14 days. Four studies including 156 subjects evaluated the effect of L-arginine supplementation on infectious complications (including pneumonia, abdominal

abscess, fasciitis, bacteremia, septic shock, septic coagulopathy, wound infections and urinary tract infections). The meta-analyses found a reduced incidence of infectious complications in the L-arginine group compared to the control group. No significant differences were seen between the groups in length of hospital stay. No other adverse effects are mentioned.

### **Risk assessment for the amino acids taurine, L-glutamine and L-arginine. Shao and Hathcock, 2008**

This review includes 38 randomised controlled clinical trials involving oral supplements of L-arginine (Shao and Hathcock, 2008). The trials included healthy adults or various patient groups. The highest dose used was 42 g/day (10 cystic fibrosis patients, 6 weeks duration), and the longest study duration was 3 years (76 adult renal transplant patients who received 9 g/day of arginine plus canola oil). Except for one study on patients with acute ST-segment elevation myocardial infarction, which was stopped due to several unexpected deaths in the intervention group (Schulman et al., 2006), no serious adverse effects were reported.

Because available data precluded the determination of a NOAEL or LOAEL, and thus also the determination of a UL, Shao and Hathcock introduced the term "observed safe level" (OSL) based on the reviewed studies. They concluded that, based on the available published human clinical trial data, the evidence for the absence of adverse effects is strong for L-arginine at intakes up to 20 g/day, and this level was therefore proposed as the OSL for normal healthy adults. They further argued that although much higher levels of L-arginine have been tested without adverse effects and may be safe, the data for intakes above these levels are not sufficient for a confident conclusion of long-term safety.

The authors chose a study by Chin-Dusting et al. (1996) on 16 healthy men as the basis for the observed safe level for supplemental L-Arginine of 20 g/day. This study tested 20 g arginine per day for 28 days and had relevant safety outcome measures. About 30 additional studies yielded results which supported 20 g/day, which was therefore proposed.

#### ***2.4.1.4 Arginine alpha-ketoglutarate***

### **Effects of 7 days of arginine-alpha-ketoglutarate supplementation on blood flow, plasma L-arginine, nitric oxide metabolites, and asymmetric dimethyl arginine after resistance exercise. Willoughby et al., 2011**

In this randomised controlled trial, 24 physically active men underwent 7 days of AAKG supplementation with 12 g/day of either "NO(2) Platinum" (trade name) or placebo (Willoughby et al., 2011). Hemodynamics, brachial-artery blood flow and circulating levels of metabolites were measured immediately before, immediately after and 30 minutes after each exercise session. Plasma L-arginine was increased with NO(2). After 7 days of supplementation, participants reported by questionnaire whether they had tolerated the supplement and reported any medical problems or symptoms they may have encountered.

None of the participants reported any side effects associated with ingesting NO(2) or placebo.

### **Pharmacokinetics, safety, and effects on exercise performance of L-arginine $\alpha$ -ketoglutarate in trained adult men. Campbell et al., 2006**

This paper reports a randomised controlled trial of 35 resistance-trained adult men who were randomly assigned to ingest 12 g of AAKG (4 g three times a day) or placebo for 8 weeks (Campbell et al., 2006). AAKG supplementation appeared to be safe and well tolerated. According to the authors, "No significant clinical side effects, related or unrelated to the study, were reported", and "All subjects tolerated the supplementation protocols without any problems".

#### ***2.4.1.5 Interactions***

Because L-arginine may lower blood pressure, antihypertensive drugs may interact with L-arginine, causing blood pressure to become too low. Medications that increase blood flow to the heart (nitrates) may also interact with L-arginine and cause dizziness and lightheadedness. Sildenafil containing drugs can lower blood pressure, and can interact with L-arginine.

#### ***2.4.1.6 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.

### **2.4.2 Animal studies**

No animal studies from the literature searches were relevant for dose-response, setting a NOAEL or the risk assessment of arginine.

A few animal studies were included in the IOM (2005) report, but none of these could be used to establish an NOAEL or an LOAEL. A brief summary of this chapter follows.

Adverse Effects in Animals. Feeding low-protein diets supplemented with 4, 5, or 7.5% arginine resulted in depressed body weight gains in rats (Harper et al., 1966; Sauberlich, 1961). However, the growth suppression by excess arginine was lessened when the protein content of the diet was increased and when the quality of protein was improved (Harper et al., 1970). Oral doses of L-arginine of 0.1, 0.5, and 1.0 g/kg bw were given to rats 1 hour before behavioral trials for a period of 5 or 7 days. Avoidance behavior was increased in Cohen Diabetic Rats (CDR) (a strain with poor learning capacity) at the highest dose only. Conditioned avoidance was not affected in Wistar rats, but increased locomotion was reported (Drago et al., 1984). Studies on the effects of orally administered arginine on the

immune system have provided conflicting results. Barbul et al. (1980) reported significant increases in thymus weights, thymic lymphocyte content, and in vitro activity of thymic lymphocytes after supplementing the diet of male mice with 0.5, 1, 2, and 3 percent arginine hydrochloride (one-half in the diet and one-half in drinking water) for 6 days. No dose–response was found, with the maximum stimulation noted at 0.5 percent supplementation of the normal chow diet containing 1.8% arginine.

Reynolds et al. (1990) reported significantly increased thymus weight, spleen cell mitogenesis, and inducible natural killer cell activity in mice after oral arginine (drinking water) doses of 60, 120, or 240 mg/kg bw per day. No dose–response was reported with maximum stimulation noted at 60 mg/kg bw per day. In young or aged rats, ingestion of diets supplemented with 3 percent L-arginine for 15 days did not result in increased thymus weights and little effect was reported on lymphocyte proliferation or interleukin-2 production as compared to controls (Ronnenberg et al., 1991).

### **2.4.3 Mode of action for adverse effects**

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

### **2.4.4 Vulnerable and high intake groups**

Gui et al. (2014) reviewed published randomised trials of arginine supplementation for improving maternal and neonatal outcomes in hypertensive disorders of pregnancy. Seven randomised controlled trials were identified, including a total of 916 women with hypertensive disorders of pregnancy. A wide range of doses were used. No effects on systolic blood pressure or neonatal weight were observed. Three of the studies reported some adverse effects, e.g. nausea, dyspepsia, vomiting and headache. No teratogenic or lethal effects were reported. The authors concluded that the risk (not defined) of arginine administration seemed uncommon.

The most serious adverse event reported in an RCT of arginine supplementation is that in the study of patients included shortly after having suffered an acute myocardial infarction. The study included 153 patients with acute ST-segment elevation myocardial infarction, who were randomised to receive 3 x 3 g/day of L-arginine (as Arg-HCl), or placebo, for 6 months (Schulman et al., 2006). No group differences in the outcome measures, vascular stiffness and ejection fraction, were found. However, 6 participants (8.6%) in the L-arginine group died versus none in the placebo group. Enrolment was stopped due to safety concerns. Because these patients were enrolled shortly after their myocardial infarction, they may have been especially vulnerable. Until more evidence becomes available, it may be appropriate to advise against arginine supplementation for this group of patients, who may be considered a vulnerable group.

No studies in children and adolescents, relevant for this risk assessment, have been identified.

## 2.5 Summary of hazard identification and characterisation

### L-Arginine

Information about potential adverse effects from arginine supplements has been derived from articles retrieved from literature searches and available information summarised in previous risk assessments.

The IOM report from 2005 concluded that since neither a NOAEL nor a LOAEL could be identified for intake of L-arginine from dietary supplements in healthy individuals, a UL could not be determined. The report did not conclude on which level of arginine supplementation that may be considered unlikely to cause adverse health effects.

The VKM report from 2011 cited the review by Shao and Hathcock (2008) who had suggested that since an NOAEL or LOAEL cannot be identified, the term observed safe level should be used and this should be 20 g/day. The report did not conclude on which level of arginine supplementation that may be considered unlikely to cause adverse health effects.

The Spanish ASEAN report from 2012 concluded that since neither a NOAEL nor LOAEL could be identified for intake of L-arginine from dietary supplements in healthy individuals, a UL could not be determined. They nevertheless proposed that a maximum daily amount of 3 g of L-arginine is acceptable from the safety point of view for use as a food supplement. However, the rationale for selecting 3 g/day was not provided.

Studies have typically been designed to test positive health effects of L-arginine, and not focused on possible negative health effects. The following, mostly minor, adverse effects of arginine supplementation have been mentioned in the articles: various gastrointestinal symptoms, endothelial function, infections, dizziness, palpitations and headaches. One study found a higher incidence of mortality among patients with acute myocardial infarction. However, reporting of adverse effects has not been systematically monitored by objective measures, but mostly been through self-reporting and clinical observations.

Several RCTs have studied the effects of supplementation of L-arginine in various patient groups and in pregnant women at risk for hypertensive disorders of pregnancy. A few small studies of short duration have included healthy individuals. The study groups have included patients with diabetes, metabolic syndrome, cardiovascular disease including peripheral artery disease, and compromised immune system. The highest dose found was 42 g/day for six weeks, in a study of 10 cystic fibrosis patients without adverse effects reported. The longest duration is 9 g/day arginine plus canola oil for 3 years, among 76 adult renal transplant patients. Of 10 studies in healthy individuals, the doses ranged from 8 to 30 g/day, study durations ranged from 7 to 28 days, and the numbers of subjects were small, from 6 to 45. In none of these studies were adverse effects reported.

The most serious adverse event reported in an RCT of arginine supplementation is that in the study of patients included shortly after having suffered an acute myocardial infarction

(Schulman et al., 2006). This study was terminated because of a higher mortality rate in the arginine arm compared to the placebo arm. The study included acutely ill patients and the results are therefore not considered to be generalizable to healthy subjects.

In all the other RCTs reviewed, no significant differences in serious adverse events between intervention and placebo groups have been reported. Most adverse effects with L-arginine were various mild gastrointestinal symptoms, mostly self-reported.

No studies in children and adolescents, relevant for this risk assessment, have been identified.

Most studies of arginine supplementation have been of short duration. Two of the reviewed RCTs of the longest duration of arginine supplementation lasted 6 and 18 months, both with a daily dose of 6.4 g of arginine. Due to their long duration, results from these two RCTs are especially relevant for this risk assessment. The most recent is the study by Monti et al. (2012) which randomised 144 patients with impaired glucose tolerance and metabolic syndrome to receive 6.4 g/day of arginine or placebo for 18 months. The study was designed to assess safety and efficacy of arginine. Adverse events were collected and reviewed during the study period, and did not differ between arginine and placebo groups.

The other study of high relevance for this risk assessment was reported by Lucotti et al. (2009). 32 patients with stable coronary artery disease, after successful coronary artery bypass grafting, were randomised to receive 6.4 g/day of arginine or placebo for 6 months. No patients manifested adverse effects during the study period.

Shao and Hathcock, in a review of the safety of arginine supplementation published in 2008, proposed an "observed safe level" of 20 g/day. However, this is not an established term used in risk assessment.

Based on the literature reviewed, it is not possible to make assumptions for potential negative health effects of supplementation with arginine for periods longer than 6 months. The two studies of the longest duration without adverse health effects used 6.4 g/day for 6 and 18 months, without causing adverse events.

As value for comparison used in the risk characterisation of L-arginine, VKM will therefore use 6.4 g/day, based on the two most relevant randomised controlled trials lasting 6 and 18 months. In an adult weighing 70 kg, 6.4 g/day of arginine corresponds to 91 mg/kg bw per day.

### **Arginine alpha-ketoglutarate**

AAKG is one of several compounds that has been used as a source of arginine in food supplements. AAKG has been studied in healthy athletic men without serious adverse side effects. However, there are too few studies of AAKG supplementation to make conclusions for this specific compound.



# 3 Exposure / Intake

Exposure of L-arginine was estimated from the intake of food supplements for the age groups 10-14 years, 14-18 years and adults (≥18 years).

## 3.1 Food supplements

The NSFA has requested a risk assessment of the intake of 3000, 3500, 4000, 4500, 5000, 5500, 6000 and 6800 mg L-arginine/day and 1000 and 2000 mg arginine alpha-ketoglutarate from food supplements for children, adolescents and adults. The default body weights (bw) for these groups determined by the EFSA were used: 10 to <14 years=43.4 kg, 14 to <18 years=61.3 kg, and adults=70 kg. The intakes per kg bw is given in (Table 3.1-1).

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

Groups	Daily doses, L-arginine, mg	Body weight	Exposures (mg/kg bw per day)
<b>Children (10 to &lt;14years)</b>	3000, 3500, 4000, 4500, 5000, 5500, 6000, 6800	43.4	69, 81, 92, 104, 115, 127, 138, 157
<b>Adolescent (14 to &lt;18 years)</b>	3000, 3500, 4000, 4500, 5000, 5500, 6000, 6800	61.3	49, 57, 65, 73, 82, 90, 98, 111
<b>Adults (≥18 years)</b>	3000, 3500, 4000, 4500, 5000, 5500, 6000, 6800	70.0	43, 50, 57, 64, 71, 79, 86, 97

Exposure of L-arginine was estimated from the intake of food supplements alone.

According to FAO/WHO/UNU, the requirements for L-arginine in adults are 117 mg/kg bw per day (WHO, 2007).

**Table 3.1-2:** Arginine alpha-ketoglutarate: an overview of the exposure of children, adolescents and adults in the present risk assessment.

<b>Groups</b>	<b>Daily doses, L-arginine, mg</b>	<b>Body weight</b>	<b>Exposures (mg/kg bw per day)</b>
<b>Children (10-14 years)</b>	1000, 2000	43.4	23.0 to 46.1
<b>Adolescent (14-18 years)</b>	1000, 2000	61.3	16.3 to 32.6
<b>Adults (&gt;18 years)</b>	1000, 2000	70.0	14.3 to 28.6

Exposure of Arginine alpha-ketoglutarate was estimated from the intake of food supplements alone.

### **Other sources**

The normal intake of an adult with a mixed protein diet is 5.4 g/100 g of mixed proteins (IOM, 2005). Based on intake distribution data from the 1988–1994 NHANES III, mean daily intakes for all life stage and gender groups of arginine from food and supplements is approximately 4.2 g/day. Men 51 through 70 years of age had the highest reported intake at the 99th percentile of 10.1 g/day (IOM, 2005).

Under normal conditions, endogenous daily production of 15-20 g arginine occurs via the citrulline intestinal-renal axis (Barbul and Uliyargoli, 2007).

# 4 Risk characterisation

## **L-arginine**

The doses received from NFSA are L-arginine: 3000, 3500, 4000, 4500, 5000, 5500, 6000 and 6800 mg/day and AAKG: 1000 and 2000 mg/day in food supplements, and the exposures for adults, adolescents and children at or above 10 years are given in chapter 3.

The value for comparison used in this risk characterisation of arginine is 6.4 g/day, corresponding to 91 mg/kg bw per day in a 70 kg adult. This is based on the doses used in the two randomised controlled trials of longest duration (six months and 18 months) with no reported negative health effects.

No studies with arginine in children were found. However, there are no data indicating that children and adolescent are more vulnerable than adults for arginine. No tolerance level is set for arginine specifically for children or adolescents. Assuming similar tolerance for these age groups as for adults, the same value for comparison as for adults are used for children and adolescents (91 mg/kg bw per day).

VKM considers that:

In adults ( $\geq 18$  years), the specified doses 3000, 3500, 4000, 4500, 5000, 5500 and 6000 mg/day L-arginine in food supplements are considered unlikely to cause adverse health effects, whereas the dose 6800 mg/day may represent a risk of adverse health effects.

In adolescents (14 to  $< 18$  years), the specified doses 3000, 3500, 4000, 4500, 5000, 5500 mg/day L-arginine in food supplements are considered unlikely to cause adverse health effects, whereas the doses 6000 and 6800 mg/day may represent a risk of adverse health effects.

In children (10 to  $< 14$  years), the specified doses 3000 and 3500 mg/day L-arginine in food supplements are considered unlikely to cause adverse health effects, whereas the doses 4000, 4500, 5000, 5500, 6000 and 6800 mg/day may represent a risk of adverse health effects.

## **Arginine alpha-ketoglutarate**

The doses requested from NFSA are 1000 and 2000 mg arginine alpha-ketoglutarate per day in food supplements. For arginine alpha-ketoglutarate there is not sufficient data to assess the risk of adverse effects for any population group.

In terms of the arginine content, a dose of 1000 mg AAKG contains 544 mg arginine and 450 mg alpha-ketoglutarate (based on the molecular weight of 174.2 g/mol for arginine and 144.1 g/mol for alpha-ketoglutarate). A dose of 2000 mg AAKG, the highest dose sold in Norway as a food supplement, contains 1088 mg arginine and 900 mg alpha-ketoglutarate.

This amount of arginine is well below the lowest specified dose of 3000 mg/day L-arginine sold in food supplements in Norway.

# 5 Uncertainties

## **L-arginine**

The total exposure of L-arginine from other sources than food supplements is not included in the risk assessment.

No relevant studies on children and adolescents were identified in the literature search making the conclusions for these groups uncertain.

The risk assessment is based on previous risk assessments of L-arginine, containing no information on vulnerable groups, interactions, allergy or mechanisms of action for adverse effects.

Several of the studies referred to are designed to investigate potential beneficial rather than harmful effects. Adverse effects are often not properly monitored and/or recorded and several studies do not mention possible adverse effects. Where adverse effects are reported in the published studies, they are mostly based on self-reported symptoms by the study participants.

There is a lack of long term human studies focusing on possible negative health effects, and it is therefore not possible to make assumptions about potential negative health effects.

## **Arginine alpha-ketoglutarate**

For AAKG there is not sufficient data to assess the risk of adverse effects for any population group. A risk assessment of alpha-ketoglutarat has not been performed as part of this report.

# 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-arginine and arginine alpha-ketoglutarate in food supplements at the following doses: L-arginine: 3000, 3500, 4000, 4500, 5000, 5500, 6000 and 6800 mg/day and arginine alpha-ketoglutarate: 1000 and 2000 mg/day for the general population, ages 10 years and above.

It is well documented that doses up to 6.4 g per day of L-arginine in adults is unlikely to cause adverse health effects in studies of up to 18 months duration. Some adverse health effects related to intermittent gastrointestinal symptoms have been reported in the reviewed literature, but apart from one study on patients with acute myocardial infarction, the symptoms have not been serious. Patients in the acute phase of a myocardial infarction may be a vulnerable group.

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

VKM concludes that:

- In adults ( $\geq 18$  years), the specified doses 3000, 3500, 4000, 4500, 5000, 5500 and 6000 mg/day L-arginine in food supplements are considered to be unlikely to cause adverse health effects, whereas the dose 6800 mg/day may represent a risk of adverse health effects.
- In adolescents (14 to  $<18$  years), the specified doses 3000, 3500, 4000, 4500, 5000, 5500 mg/day L-arginine in food supplements are considered to be unlikely to cause adverse health effects, whereas the doses 6000 and 6800 mg/day may represent a risk of adverse health effects.
- In children (10 to  $<14$  years), the specified doses 3000 and 3500 mg/day L-arginine in food supplements are considered to be unlikely to cause adverse health effects, whereas the doses 4000, 4500, 5000, 5500, 6000 and 6800 mg/day may represent a risk of adverse health effects.
- No dosage of arginine alpha-ketoglutarate as a single substance in food supplements can be evaluated due to lack of data.

Overviews of the conclusions are presented in Tables 6.1 and 6.2.

**Table 6.1:** An overview of the conclusions for L-arginine in food supplements.  
 Green: Estimated exposures to L-arginine are unlikely to cause adverse health effects.  
 Red: Estimated exposures to L-arginine may represent a risk of adverse health effects.

		<b>L-arginine</b>							
Doses		3000	3500	4000	4500	5000	5500	6000	6800
Age groups		mg/day	mg/day	mg/day	mg/day	mg/day	mg/day	mg/day	mg/day
Children (10 to <14 years)		Green	Green	Red	Red	Red	Red	Red	Red
Adolescents (14 to <18 years)		Green	Green	Green	Green	Green	Green	Red	Red
Adults (≥18 years)		Green	Green	Green	Green	Green	Green	Green	Red

**Table 6.2:** An overview of the conclusions for arginine alpha-ketoglutarate in food supplements.  
 Grey: No conclusion can be made.

		<b>Arginine alpha-ketoglutarate</b>	
Doses		1000 mg/day	2000 mg/day
Age groups			
Children (10 to <14 years)		No conclusion	No conclusion
Adolescents (14 to <18 years)		No conclusion	No conclusion
Adults (≥18 years)		No conclusion	No conclusion

# 7 Data gaps

## **L-arginine**

There is a lack of studies of adverse effects as primary outcomes of L-arginine in humans. The studies which have reported negative health effects related to L-arginine in adults have high heterogeneity both in design, target population, and results.

No information on negative health effects/adverse effects of L-arginine in children or adolescents was reported in the included previous risk assessments.

There is lack of acute, sub-chronic and chronic toxicity studies of sufficient quality.

There are no studies on negative health effects related to L-arginine in children and adolescents. L-arginine has been given to pregnant women without serious adverse effects.

## **Arginine alpha-ketoglutarate**

No previous published reports have reviewed AAKG as a food supplement. There is not sufficient information to draw a conclusion regarding the potential risk of taking supplements containing AAKG.

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# Appendix 1

## Search strategies for this risk assessment

### Search strategy for human studies

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

1. arginine\*.ti. (36733)
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. (8930085)
3. 1 and 2 (6687)
4. (conference abstract\* or letter\* or editorial\*).pt. (4374047)
5. 3 not 4 (6355)
6. limit 5 to (danish or english or norwegian or swedish) (6146)
7. limit 6 to human (2344)
8. remove duplicates from 7 (1362)

### Search strategy for arginine alpha-ketoglutarate

Database: Embase <1974 to 2015 September 10>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily, Ovid MEDLINE(R) and Ovid OLDMEDLINE(R) <1946 to Present>

1. ketoglutarate\*.ti. (2479)
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. (9460797)
3. 1 and 2 (310)
4. (conference abstract\* or letter\* or editorial\*).pt. (4714936)
5. 3 not 4 (293)
6. limit 5 to (danish or english or norwegian or swedish) (277)
7. remove duplicates from 6 (155)

### Search strategy for animal studies

Database: Embase <1974 to 2015 November 10>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily, Ovid MEDLINE(R) and Ovid OLDMEDLINE(R) <1946 to Present>

1. arginine\*.ti. (38179)
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. (9592045)

3. 1 and 2 (7077)
4. (conference abstract\* or letter\* or editorial\*).pt. (4801265)
5. 3 not 4 (6713)
6. limit 5 to (danish or english or norwegian or swedish) (6499)
7. limit 6 to animals (2306)
8. remove duplicates from 7 (1500)