



VKM Report 2016: 24

# Risk assessment of "other substances" – L-histidine

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

Report from the Norwegian Scientific Committee for Food Safety (VKM) 2016: 24  
Risk assessment of "other substances" – L-histidine

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Norwegian Scientific Committee for Food Safety  
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ISBN: 978-82-8259-214-7  
Norwegian Scientific Committee for Food Safety (VKM)  
Po 4404 Nydalen  
N – 0403 Oslo  
Norway

Phone: +47 21 62 28 00

Email: [vkm@vkm.no](mailto:vkm@vkm.no)

[www.vkm.no](http://www.vkm.no)

[www.english.vkm.no](http://www.english.vkm.no)

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## **Risk assessment of "other substances" - L-histidine**

### **Authors preparing the draft opinion**

Kristin Holvik, Per Ole Iversen and Arild Vakt skjold.

(In alphabetic order)

### **Assessed and approved**

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

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

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### **Competence of VKM experts**

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

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# Summary

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

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

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

According to information from NFSA, L-histidine is an ingredient in food supplements and energy drinks sold in Norway. NFSA has requested a risk assessment of 550 and 600 mg/day of L-histidine from food supplements. The recommended dietary allowance (RDA) for adults of L-histidine is 14 mg/kg body weight/day (IOM, 2005), which corresponds to 980 mg/day for a 70 kg person. Oral doses has a bioavailability of 80% or higher. Foods rich in histidine are generally protein rich foods such as meat, dairy products, legumes, fish, nuts, seeds, eggs and whole grains. Based on NHANES III (1988-1994), the overall mean intake of L-histidine from food and food supplements in the United States was 2.2 g/day.

L-histidine is a conditionally essential amino acid which is a normal constituent of most body proteins. L-histidine is also a part of many plasma proteins. It has anti-oxidant and anti-inflammatory properties. Moreover, L-histidine is also a precursor of histamine and is necessary for the regulation and metabolism of trace elements such as metal ions. The human body has a large pool of L-histidine in plasma proteins, but also as carnosine in skeletal muscles and in haemoglobin.

Due to the lack of adequate scientific information, a no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL) have not been identified, and a tolerable upper intake level for histidine has not been established. Effects of histidine supplementation have been studied in trials with duration of up to 3-4 months. Previous risk assessments concluded that supplementation with 4.0 to 4.5 g/day of L-histidine above the dietary content does not have adverse effects in human beings, and new data retrieved in the present literature search were in accordance with these conclusions. No particular population groups have been identified as particularly susceptible to adverse effects of consuming histidine supplements. We have not identified any studies in children or adolescents.

VKM concludes that:

- In adults ( $\geq 18$  years), the specified doses 550 and 600 mg/day L-histidine in food supplements are unlikely to cause adverse health effects.
- In adolescents (14 to  $< 18$  years), the specified doses 550 and 600 mg/day L-histidine in food supplements are unlikely to cause adverse health effects.
- In children (10 to  $< 14$  years), the specified doses 550 and 600 mg/day L-histidine in food supplements are unlikely to cause adverse health effects.

Children younger than 10 years were not within the scope of the present risk assessment.

### **Short summary**

At the request of the Norwegian Food Safety Authority, the Norwegian Scientific Committee for Food Safety (VKM) has characterised the risk of specified doses of L-histidine in food supplements. VKM concludes that:

- In adults ( $\geq 18$  years), the specified doses 550 and 600 mg/day L-histidine in food supplements are unlikely to cause adverse health effects.
- In adolescents (14 to  $< 18$  years), the specified doses 550 and 600 mg/day L-histidine in food supplements are unlikely to cause adverse health effects.
- In children (10 to  $< 14$  years), the specified doses 550 and 600 mg/day L-histidine in food supplements are unlikely to cause adverse health effects.

**Key words:** Histidine, food supplement, adverse health effect, negative health effect, Norwegian Food Safety Authority, Norwegian Scientific Committee for Food Safety, other substances, risk assessment, VKM.

# Sammendrag på norsk

På oppdrag for Mattilsynet har Vitenskapskomiteen for mattrygghet (VKM) vurdert risiko ved tilsetning av «andre stoffer» i kosttilskudd og energidrikk som selges i Norge. VKM har risikovurdert ulike bruksdoser oppgitt fra Mattilsynet. Disse risikovurderingene vil gi Mattilsynet vitenskapelig grunnlag for å regulere "andre stoffer" i kosttilskudd.

"Andre stoffer" er beskrevet i kosttilskudddirektivet (2002/46/EF) som stoffer som har en ernæringsmessig eller fysiologisk effekt, og som ikke er vitaminer og mineraler. De tilsettes i hovedsak til kosttilskudd, men også til energidrikker og andre næringsmidler. I disse risikovurderingene har VKM ikke vurdert potensielle gunstige helseeffekter, men kun vurdert mulige negative helseeffekter.

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

Ifølge informasjon fra Mattilsynet er L-histidin en ingrediens i kosttilskudd som selges i Norge. Oppdraget fra Mattilsynet var å risikovurdere følgende doser av L-histidin i kosttilskudd: 550 og 600 mg/dag. Det anbefalte daglige inntaket av L-histidin for voksne gjennom kosten er 14 mg/kg kroppsvekt per dag (IOM, 2005), som tilsvarer 980 mg/dag for en 70 kg person. Kroppens opptak av L-histidin tatt inn oralt er 80 % eller høyere. Eksempler på matvarer med et høyt innhold av histidin er proteinrike matvarer som kjøtt, meieriprodukter, bønner, fisk, nøtter, frø, egg og helkorn. Ifølge data fra NHANES III (1988-1994) var gjennomsnittlig inntak av L-histidin fra mat og tilskudd i USA 2,2 g/dag.

L-histidin er en betinget essensiell aminosyre, og er en bestanddel i de fleste proteiner i kroppen. L-histidin inngår også i mange plasmaproteiner. L-histidin har antioksidative og antiinflammatoriske egenskaper, og er dessuten en forløper til histamin og er nødvendig for regulering og metabolisme av sporstoffer, bl.a. metallioner. Menneskekroppen har et stort lager av L-histidin i plasmaprotein, dessuten er L-histidin en bestanddel i hemoglobin og i form av karnosin i muskler.

Det er ikke blitt etablert en NOAEL (no observed adverse effect level) eller LOAEL (lowest observed adverse effect level), og heller ikke et øvre tolerabelt inntaksnivå (UL) for L-histidin. Effekten av histidin er undersøkt i studier med opptil 3-4 måneders varighet. Tidligere risikovurderinger har konkludert med at tilskudd på 4,0 til 4,5 g pr dag av L-histidin utover det som inntas i den daglige kosten ikke er forbundet med negative helseeffekter hos mennesker. Nye data innhentet via litteratursøket var i samsvar med disse konklusjonene. Ingen befolkningsgrupper har blitt identifisert som særskilt utsatte for økt risiko for negative helseeffekter fra kosttilskudd med L-histidin. Det ble ikke identifisert noen studier med barn eller ungdom.



Vitenskapskomiteen for mattrygghet (VKM) konkluderer med at:

- For voksne ( $\geq 18$  år) er det usannsynlig at de spesifiserte dosene på 550 og 600 mg/dag L-histidin i kosttilskudd vil forårsake negative helseeffekter.
- For ungdom (14 til  $< 18$  år) er det usannsynlig at de spesifiserte dosene på 550 og 600 mg/dag L-histidin i kosttilskudd vil forårsake negative helseeffekter.
- For barn (10 til  $< 14$  år) er det usannsynlig at de spesifiserte dosene på 550 og 600 mg/dag L-histidin i kosttilskudd vil forårsake negative helseeffekter.

Barn under 10 år inngår ikke i dette oppdraget.

### **Kort sammendrag**

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

- For voksne ( $\geq 18$  år) er det usannsynlig at de spesifiserte dosene på 550 og 600 mg/dag L-histidin i kosttilskudd vil forårsake negative helseeffekter.
- For ungdom (14 til  $< 18$  år) er det usannsynlig at de spesifiserte dosene på 550 og 600 mg/dag L-histidin i kosttilskudd vil forårsake negative helseeffekter.
- For barn (10 til  $< 14$  år) er det usannsynlig at de spesifiserte dosene på 550 og 600 mg/dag L-histidin i kosttilskudd vil forårsake negative helseeffekter.

# Abbreviations and glossary

## Abbreviations

AESAN	- Spanish Agency for Food Safety and Nutrition
bw	- body weight
BMI	- body mass index
CoA	- co-enzyme A
EFSA	- European Food Safety Authority
IOM	- Institute of Medicine, USA
LOAEL	- lowest observed adverse effect level
NFSA	- Norwegian Food Safety Authority [ <i>Norw.</i> : Mattilsynet]
NOAEL	- no observed adverse effect level
RCT	- randomised controlled trial
UL	- tolerable upper intake level
VKM for Mattrygghet]	- 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. The World Health Organization (WHO) has established the following definition of "adverse effect": a change in morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences (WHO, 1994).

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

# Background as provided by the Norwegian Food Safety Authority

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

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

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

The Norwegian Food Safety Authority (NFSA) is working on the establishment of a regulation on the addition of "other substances" to foods at a national level. The regulation will include a list of substances with permitted maximal doses, based on the substances and doses found in products on the Norwegian market. In preparation for a regulation, NFSA has therefore requested the Norwegian Scientific Committee for Food Safety (VKM) to assess the safety of "other substances" found on the Norwegian market. NFSA, in consultation with the industry, has compiled a list of "other substances" found in products marketed in Norway. Only substances with a purity of minimum 50% or concentrated 40 times or more have been included in the list. Substances regulated by other legislations like those for novel foods, food additives, aromas, foods for special medical purposes, etc. have been excluded from the list.

# Terms of reference as provided by the Norwegian Food Safety Authority

The Norwegian Food Safety Authority (NFSA) requested the Norwegian Scientific Committee for Food Safety (VKM) to assess the safety of L-histidine in food supplements at the following doses: 550 and 600 mg/day.

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

The safety assessments for "other substances" present in food supplements shall be carried out for the general population, age 10 years and older.

# 1 Introduction

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

This risk assessment regards the substance L-histidine per se, and no specific products.

In this series of risk assessments of "other substances" VKM has not evaluated any claimed beneficial effects from these substances, but merely possible adverse effects at specified doses used in Norway.

According to information from the Norwegian Food Safety Authority (NFSA), histidine is an ingredient in food supplements sold in Norway. NFSA has requested a risk assessment of the intake of 550 and 600 mg L-histidine per day from food supplements. The total histidine exposure from other sources than food supplements is not included in the risk assessment.

Adults, in contrast to children, can partly meet their needs for L-histidine by endogenous synthesis. This amino acid is abundant in haemoglobin and in muscle- and plasma proteins (Christman, 1971). L-histidine is a precursor of histamine, and it is part of ion-binding proteins critical for muscular contraction and iron-binding proteins responsible for oxygen transport. L-histidine has anti-oxidant and anti-inflammatory properties (Wade and Tucker, 1998). It functions as a free radical scavenger and chelates divalent metal ions, and is necessary for the regulation and metabolism of trace elements such as zinc, copper, iron, manganese and molybdenum.

Foods rich in histidine are generally protein rich foods such as meat, dairy products, legumes, fish, nuts, seeds, eggs and whole grains. The requirements for adults of L-histidine are 10 mg/kg bw per day (WHO, 2007), corresponding to 700 mg/day for a 70 kg adult. The recommended dietary allowance (RDA) of L-histidine for adults is 14 mg/kg bw per day (IOM, 2005), corresponding to 980 mg/day for a 70 kg adult. According to NHANES III (1988-1994), the overall mean intake of L-histidine from food and food supplements in the United States was 2.2 g/day. Men aged 51-70 years had the highest intakes at the 99th percentile of 5.2 g/day (IOM, 2005).

# 2 Hazard identification and characterisation

## 2.1 Literature

This risk assessment is based on previous risk assessments of L-histidine, as well as scientific papers retrieved from a systematic search in literature published in the period 1 January 2012 – 27 October 2015 in order to retrieve any recent studies on adverse effects caused by L-histidine published after the search included in the VKM report from 2013.

### 2.1.1 Previous risk assessments

Risks related to L-histidine have previously been evaluated by the Institute of Medicine (IOM) in USA in 2005, the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (ASEAN) for use in food supplements in 2012 and 2013, and by VKM in 2013 (AESAN, 2012; AESAN, 2013; IOM, 2005; VKM, 2013).

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

The report concluded that available scientific data are not adequate to derive an upper tolerable intake level (UL) for the chronic oral intake of L-histidine from supplements, and that there is evidence in humans that doses of L-histidine between 4 and 4.5 g/day above the amounts found in the diet do not result in adverse effects (IOM, 2005). However, the evidence was considered tentative as the number of individuals studied was low and the studies lacked dose-response information. Studies on animals and humans suggest that intake of high doses of histidine may alter copper and zinc metabolism. Supplementation with about 4 g/kg bw per day in rats and 8 to 65 g/day in humans has an impact on serum zinc concentration by increasing urinary excretion of zinc, with possible zinc deficiency as a result. However, the human data is based on six patients with systemic progressive sclerosis representing a particular patient population (Henkin et al., 1975). Blumenkrantz et al. (1975) found that 4 g of histidine supplement/day for 17.5 weeks to humans did not cause adverse effects.

A feeding study in rats reported significantly reduced concentrations of copper and zinc in the plasma and reduced liver concentrations of copper after feeding diets containing 8% L-histidine (about 4 g/kg bw per day) for 46 days (Harvey et al., 1981). In a small group of newborn infants who received total parenteral nutrition, 165 mg of L-histidine per kg bw per day caused an increased urinary excretion of zinc compared with 95 mg of L-histidine per kg

bw per day when studied for an initial adaptation period of 72 hours followed by 2 x 48 hours of urine sampling (Zlotkin, 1989).

In rats, short-term feeding studies (7 to 46 days) have shown growth retardation and hepatomegaly at L-histidine levels of approximately 2 to 4 g/kg bw per day. No carcinogenicity, neoplastic changes or sperm granuloma have been observed in rats. Only one chronic dose-response study in rats was identified by the authors of the report. In this study, Fischer 344 rats were fed 0, 1.25% and 2.5% L-histidine monohydrochloride for 104 weeks. No statistically significant increase in the incidence of any tumor was found in the treated groups of either sex (Ikezaki et al., 1996).

The literature search was not described.

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

The use of L-histidine as a food supplement was assessed. In line with (IOM, 2005), AESAN (2012) cited the rat feeding studies where delayed growth, hepatomegaly and hypercholesterolemia, but no carcinogenicity or sperm granuloma, had been observed (doses 2 and 4 g/kg bw per day). They also cited the studies in both animals (about 4 g/kg bw per day) and humans (8 to 65 g/day) suggesting that high histidine intake may influence serum zinc concentration and urinary excretion, with zinc deficiency as a possible result. Animal studies also indicated that high histidine levels may decrease copper levels. (AESAN, 2012) stated that the available evidence suggests that 4.0 to 4.5 g/day of L-histidine above the dietary intake do not have adverse effects in humans, and a maximum daily amount of 750 mg of L-histidine was concluded acceptable as supplement. A rationale for the maximum daily amount of 750 mg supplemental histidine was not provided in the report.

The literature search was not described. The cited studies were published in the period 1971-2010.

***Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on the use conditions for certain substances other than vitamins, minerals and plants in food supplements-2. Spain, 2013***

The (AESAN, 2012) report was updated in 2013, evaluating a higher dose of L-histidine from supplements based on the authorised level in Italy. Based on the same evidence as previously, the AESAN (2013) concluded that a maximum daily quantity of 1.12 g of L-histidine as food supplement is acceptable.

The literature search was not described. The included sources were published in the period 1972-2012.

## ***Risk assessment of histidine, methionine, S-adenosylmethionine and tryptophan (VKM). Norway, 2013***

Potential adverse health effects of the use of L-histidine as a food supplement were assessed. No new studies of adverse health effects after the IOM (2005) report was identified, and no UL was suggested. VKM (2013) supported the IOM (2005) conclusion that doses at 4 to 4.5 g/day had not resulted in adverse effects. The main concern was that elevated doses of L-histidine may alter copper and zinc metabolism. No particular population groups were identified as having high risk from L-histidine supplementation. The request from the Norwegian Food Safety Authority concerned amino acid powders available on the Norwegian market that contained up to 2.5 g histidine per recommended daily dosage. The report concluded that supplements sold in Norway containing 77 mg to 2.5 g L-histidine per daily dosage should not be of concern.

Details about the literature search were presented (time period from 2002 to October 2012).

### **2.1.2 Literature search**

The literature search for the present report was performed in MEDLINE and EMBASE in order to retrieve publications from 2012 to present on adverse effects caused by L-histidine. The year 2012 was included to obtain overlap with the evidence base available for the most recent reports published in 2013. Both databases were searched to ensure comprehensive study retrieval. The literature search was conducted 23 October 2015, and was designed to obtain both human and animal studies. Since studies in children and adolescents were lacking, an additional literature search for these groups was conducted. The additional search specified for children and adolescents was performed 2 December 2015. The strategies for the searches are outlined in Appendix 1.

#### ***2.1.2.1 Publication selection and data extraction***

The first literature search identified 329 publications. In the primary screening, titles and abstracts of all unique publications retrieved were independently screened against the inclusion criteria.

Inclusion criteria:

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

In vitro studies were not included.



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

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

The first screening resulted in eight full text articles, while the secondary screening resulted in one relevant article. Additionally, one study from manual search was identified and reviewed. Only one human study was found relevant and included in the results in this report (see Figure 2.1.2.1-1). No new articles were found in the literature search specified for children and adolescents, and no new animal studies were identified.

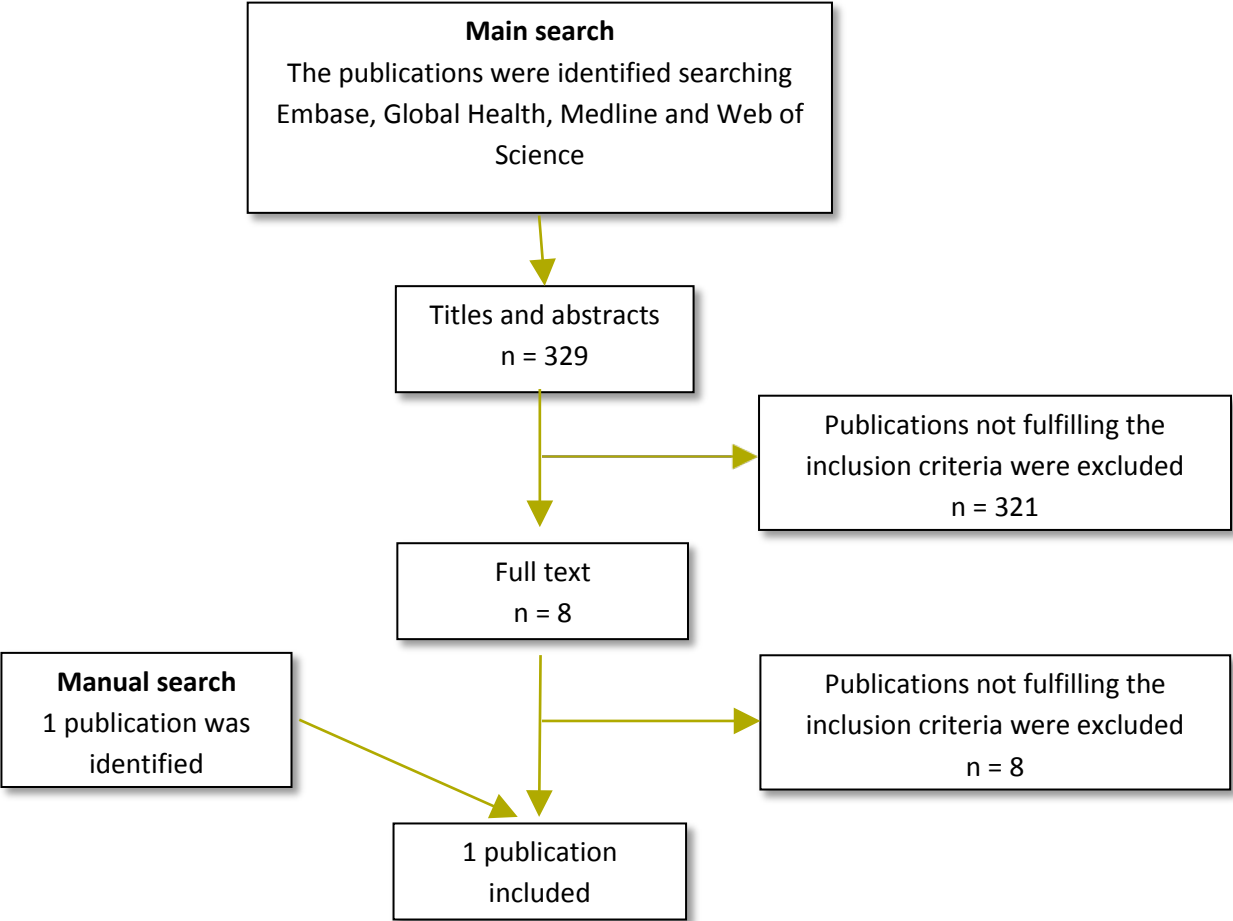
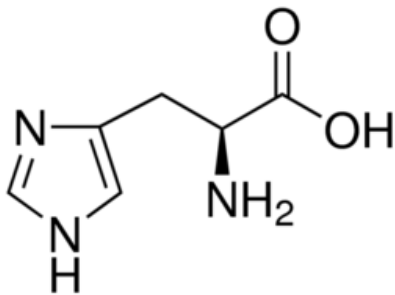


Figure 2.1.2.1-1: Flowchart for publication selection for L-histidine.

## 2.2 General information

### 2.2.1 Chemistry

L- histidine, or 2-Amino-3-(1*H*-imidazol-4-yl) propanoic acid, is a semi-essential amino acid. Water solubility: 41.6 g/L (25 °C). The CAS number for L-histidine is 71-00-1. The structural formula is shown in figure 2.2.1-1.



**Figure 2.2.1-1:** Structural formula of L-histidine

### 2.2.2 Occurrence

Histidine is not available as a free amino acid in foods, except for in human breast milk (Baldeon et al., 2014), but as a component in food protein. Rich food sources of L-histidine are e.g. game, soy protein, egg white, cod, beef, pork and grain. L-histidine is also available in food supplements.

## 2.3 Absorption, distribution, metabolism and excretion

### 2.3.1 In humans

Dietary histidine is made available to the human organism after digestion in the small intestine of the protein in which it is bound. The amino acid is absorbed as free amino acid or bound in small peptides by specific transporters in the intestinal mucosa. Histidine passes from the intestine to the liver where it is used as precursor for synthesis of plasma proteins and released into the blood circulation.

The human body has a large pool of L-histidine in plasma and muscle proteins, but also in carnosine ( $\beta$ -alanyl-L-histidine) in skeletal muscles and in haemoglobin. The plasma concentration of histidine is influenced by the diet (Samman et al., 2014). Oral doses have a bioavailability of 80% or higher (Wade and Tucker, 1998).

The plasma concentrations and clearance are similar for L-histidine administered orally and intravenously, and the peak plasma concentration occurs about one hour after exposure (Wade and Tucker, 1998). Histidine, together with glutamine, glutamate, arginine and

proline, is converted to  $\alpha$ -ketoglutarate, succinyl CoA, fumarate, and oxaloacetate in the tricarboxylic acid cycle. Histidine is deaminated into ammonia and urocanic acid by the enzyme histidine ammonia-lyase, also called histidase or histidinase, which occurs in the liver and skin. A deficiency in this enzyme is present in the rare metabolic disorder histidinemia, which leads to accumulation of histidine in plasma and tissues. Also humans with rare genetic loss-of-function variants of histidase have elevated levels of histidine (Yu et al., 2015).

Histidine is a precursor for histamine and carnosine biosynthesis, and converted to histamine by the enzyme histidine decarboxylase. Histidine is also an important component in haemoglobin (8%). L-histidine has anti-oxidant and anti-inflammatory properties. An important function of histidine is the ability to bind to various divalent metal ions (e.g. zinc, copper, iron, manganese and molybdenum (Sarkar, 1987). High levels of histidine may therefore theoretically cause deficiencies of the free forms of these metal ions. However, studies addressing the interplay between histidine and the levels of these metals have produced variable data (Vanwouwe et al., 1990).

Histidine in the form of 3-methylhistidine is present in actin and myosin (Young and Munro, 1978), but the role of methylated histidine in the function of these contractile proteins is unknown.

Histidine is excreted in both the feces and the urine. The excretion is dependent on nutritional status as malnutrition reportedly blocks fecal excretion (Antener et al., 1983).

## 2.4 Toxicological data/Adverse effects

### 2.4.1 Human studies

The literature search did not identify any relevant studies in children. Only one recent publication reporting a histidine supplementation study in adults was identified.

#### **Histidine supplementation improves insulin resistance through suppressed inflammation in obese women with the metabolic syndrome: A randomised controlled trial. Feng et al., 2013**

Ninety-two women aged 33-51 years with a body mass index (BMI)  $\geq 28$  kg/m<sup>2</sup> completed a randomised double blinded placebo-controlled trial to evaluate the efficacy of histidine supplementation on insulin resistance, inflammation, oxidative stress and metabolic disorders in obese women with metabolic syndrome (Feng et al., 2013). Forty-seven women received tablets containing 4 g of L-histidine/day for 12 weeks, while 45 women received identical placebo tablets. The findings indicated that the histidine supplement did not influence kidney or liver functions. No side effects were observed, and no complaints of headache, weakness, drowsiness, nausea or decreased appetite were reported in any of the two groups. Eight participants (8.0%) did not complete the study because of loss to follow-up, pregnancy, or not following the protocol. Excretion of metal ions in serum or urine was not measured.

#### **2.4.1.1 Interactions**

Although it is well established that histidine can bind to various divalent metal ions and thereby possibly modify the levels of these metal ions, experimental data are conflicting as to whether the metal ion concentrations increase, decrease or remain unchanged (AESAN, 2012; Henkin et al., 1975; IOM, 2005; Zlotkin, 1989). Factors that may influence the histidine-metal ion relationship include e.g. species, histidine dose, nutritional status and duration of feeding histidine.

#### **2.4.1.2 Allergic sensitisation (including adjuvant effects)**

There was no information concerning allergic sensitisation or allergy adjuvant effects in the literature reviewed in the present risk assessment. 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**

According to previous reports, animal studies have shown that high oral doses ( $\geq 2000$  mg/kg bw per day) of L-histidine for 7-46 days may cause retarded growth, hepatomegaly and hyper-cholesterolemia (Harvey et al., 1981; Hitomiomura et al., 1992; Ohmura et al., 1986; Solomon and Geison, 1978). No new animal studies were identified in the present literature search.

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

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

An important function of histidine is the ability to bind to various divalent metal ions (e.g. zinc, copper, iron, manganese and molybdenum (AESAN, 2012). High levels of histidine may therefore theoretically cause deficiencies of the free forms of these metal ions due to increased excretion.

#### **2.4.4 Vulnerable groups**

No vulnerable groups to excess doses of L-histidine have been reported. There have been no reported studies in children, elderly, pregnant women or lactating women.

### **2.5 Summary of hazard identification and characterisation**

The literature search for the VKM (2013) risk assessment of four amino acids including histidine did not identify any new human or animal studies reporting adverse health effects of histidine in the period 2002 to 2012. Hence, the risk assessments by IOM (2005), VKM (2013) and AESAN (2012) were based on the same pool of reported human studies. The

IOM (2005) and AESAN (2012) summarised that 4-4.5 g of supplemental histidine per day has not shown adverse health effects in humans, and VKM (2013) supported this conclusion.

No treatment related carcinogenicity was observed in rats that were fed 960 mg L-histidine monohydrochloride/kg bw per day for 104 weeks (Ikezaki et al., 1996).

Only one reported study in humans was identified in our literature search (Feng et al., 2013), which was a relatively large study of high methodological quality that did not observe any adverse effects of 4 g of histidine supplement per day for twelve weeks in obese women. The finding is in line with with an older study of 4 g of histidine supplement/day for 17.5 weeks (Blumenkrantz et al., 1975).

The reported findings of increased urinary zinc excretion and symptoms of zinc deficiency in six patients with progressive systemic sclerosis receiving L-histidine supplementation in doses of between 8 and 65 g/day have been pointed out by previous reports. Although it is well established that histidine can bind to various divalent metal ions and thereby possibly modify the levels of these metal ions, experimental data are conflicting as to whether the metal ion concentrations increase, decrease or remain unchanged (AESAN, 2012; Henkin et al., 1975; IOM, 2005; Zlotkin, 1989). Factors that influence the histidine-metal ion relationship include e.g. species, histidine dose, nutritional status and duration of feeding histidine.

No particular population groups have been reported as being particularly vulnerable to potential adverse health effects from histidine supplements. No relevant studies in children have been identified, although the previous risk assessments reported one study that involved 14 newborns. The newborn infants who received 165 mg of L-histidine per kg bw/day via total parenteral nutrition had a higher urinary excretion of zinc than the control group who received 95 mg of L-histidine per kg bw per day (Zlotkin, 1989), but the study was considered to have methodological shortcomings (AESAN, 2013)

For the present risk characterisation we will use a value for comparison corresponding to the dose which has not caused adverse effects in human studies, i.e. 4 g/day (corresponding to 57 mg/kg bw per day in a 70 kg adult).

# 3 Exposure / Intake

Exposure of histidine was estimated from the intake of food supplements. For food supplements, the intake was estimated for the age groups 10 to <14 years, 14 to <18 years and adults (≥18 years).

## 3.1 Food supplements

The Norwegian Food Safety Authority requested VKM to perform a risk assessment of 550 and 600 mg/day of histidine in food supplement for children (10 – 17 years) and adults. The default body weights for age groups determined by EFSA were used: 10 to <14 years = 43.4 kg, 14 to <18 years = 61.3 kg and adults = 70.0 kg. The exposures per kg bw are given in Table 3.1-1.

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

Groups	Daily doses (mg)	Body weight (kg)	Exposures (mg/kg bw per day)
Children (10 to <14 years)	550 and 600	43.4	13 and 14
Adolescents (14 to <18 years)	550 and 600	61.3	9 and 10
Adults (≥18 years)	550 and 600	70.0	8 and 9

## 3.2 Other sources

Based on NHANES III (1988-1994), the overall mean intake of L-histidine from food and food supplements in the United States was 2.2 g/day. Men aged 51-70 years had the highest intakes at the 99th percentile of 5.2 g/day (IOM, 2005).

## 4 Risk characterisation

The doses received from NFSA for assessment were 550 and 600 mg/day L-histidine in food supplements, and the estimated exposures for adults, adolescents and children 10 years and older derived from these two dose levels are given in chapter 3.

The value for comparison used in this risk characterisation is 4 g/day, corresponding to 57 mg/kg bw per day in a 70 kg adult. This is based on studies in humans with a duration of 3 to 4 months.

There have been concerns about the possible decrease in the body levels of certain divalent metal ions (e.g. copper and zinc), following increased histidine intake. However, VKM considers the evidence to be insufficient to draw such a conclusion based on available scientific data.

Our literature review did not reveal any studies of histidine in children or adolescents, and there were no studies in children 10 years or older included in previous risk assessments. However, there are no data indicating that children and adolescent are more vulnerable than adults for histidine. No tolerance level is set for histidine 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 (57 mg/kg bw per day).

Based on the estimated exposure per kg body weight in adults, adolescents and children (Table 3.1-1), VKM considers that the specified doses 550 and 600 mg/day L-histidine in food supplements are considered unlikely to cause adverse health effects in all age groups covered by the terms of reference.

## 5 Uncertainties

The literature search was not described in the IOM (2005) and (AESAN, 2012; AESAN, 2013) reports. Thus, it is not clear how well the assessments covered the published studies of L-histidine. Furthermore, it is also not clear to which extent the summaries and conclusions in each of the previous reports were based on the earlier published reports. The VKM (2013) report did include a description of the literature search, however the search was restricted back in time to 2002. The search in the present risk characterisation was limited to scientific studies published from 2012 (to overlap VKM-2013's search). Thus, relevant studies may have been missed in the current report.

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

The risk assessment is to a large degree based on previous risk assessments of L-histidine, with scarce information on vulnerable groups, interactions, allergy or mechanisms of action for adverse effects.

Few studies were 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.



## 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-histidine in food supplements at the doses 550 and 600 mg/day for the general population, ages 10 years and above.

In adults, there is documentation that doses of 4 g per day of L-histidine are unlikely to cause adverse health effects for use up to 3-4 months.

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

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

VKM concludes that:

In adults ( $\geq 18$  years), the specified doses 550 and 600 mg/day L-histidine in food supplements are considered unlikely to cause adverse health effects.

In adolescents (14 to  $< 18$  years), the specified doses 550 and 600 mg/day L-histidine in food supplements are considered unlikely to cause adverse health effects.

In children (10 to  $< 14$  years), the specified doses 550 and 600 mg/day L-histidine in food supplements are considered unlikely to cause adverse health effects.

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

**Table 7-1:** An overview of the conclusions for L-histidine in food supplements.  
 Green: Estimated exposures to L-histidine are unlikely to cause adverse health effects.

Doses	L-histidine	
	550 mg/day	600 mg/day
Age groups		
Children (10 to <14 years)		
Adolescents (14 to <18 years)		
Adults (≥18 years)		

# 7 Data gaps

There is a lack of studies of adverse effects as primary outcomes of supplemental L-histidine in humans, both for chronic and sub-chronic intakes, including studies with robust designs such as randomised trials.

Human metabolic studies are called for to elucidate the clinical relevance of potential mechanisms of action, e.g. related to the interplay between histidine and divalent cations.

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# Appendix 1

## Search strategies for this risk assessment

### Search strategy main search

Database: Embase <1974 to 2015 October 23>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily, Ovid MEDLINE(R) and Ovid OLDMEDLINE(R) <1946 to Present>

1. histidin\*.ti. (18889)
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. (9659922)
3. 1 and 2 (3467)
4. (conference abstract\* or letter\* or editorial\*).pt. (4769328)
5. 3 not 4 (3378)
6. limit 5 to (danish or english or norwegian or swedish) (3265)
7. limit 6 to yr="2012 -Current" (608)
8. remove duplicates from 7 (329)

### Search strategy for studies in children and adolescents

Database: Embase <1974 to 2015 December 02>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily, Ovid MEDLINE(R) and Ovid OLDMEDLINE(R) <1946 to Present>

1. histidin\*.ti. (18949)
2. (child\* or adolescent\* or teenage\* or college\* or high school).tw. (2963958)
3. 1 and 2 (138)
4. limit 3 to (danish or english or norwegian or swedish) (111)
5. remove duplicates from 4 (70)