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VKM Report 2023: 13

Risk assessment of hyaluronic acid in food supplements

Scientific Opinion of the Panel on nutrition, dietetic products and novel food of the Norwegian Scientific Committee for Food and Environment

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Risk assessment of hyaluronic acid in food supplement

Preparation of the opinion

The Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) appointed a project group to draft the opinion. The project group consisted of three VKM members and two VKM staff. The Committee, by the Panel on nutrition, dietetic products, novel food, and allergy, assessed and approved the final opinion.

Authors of the opinion

The authors have contributed to the opinion in a way that fulfils the authorship principles of VKM (VKM, 2019). The principles reflect the collaborative nature of the work, and the authors have contributed as members of the project group appointed specifically for the assignment.

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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

Hyaluronic acid (HA) is a high-molecular-mass polysaccharide, that is endogenously produced and present in connective tissue, synovial fluid, intraoccular fluid, and skin. The basic unit of the HA polymer consists of D-glucuronic acid and N-acetyl-D-glucosamine.

The Norwegian Food Safety Authority has requested VKM to evaluate the risk of adverse effects related to daily intake of food supplements with 150 mg, 120 mg, 64 mg, and 48 mg HA. Exposure from other sources of HA (food, cosmetics, and different medical applications) is not estimated.

HA for consumption is extracted from rooster comb and chicken cartilage or produced by bacterial fermentation. Rooster comb extract has been evaluated by European Food Safety Authority in 2013 as a novel food ingredient and found to be safe for consumption at a daily dose of 80 mg extract with a HA content between 60-80%, which translates to 48-64 mg HA per dose.

The body's own production of HA is around 5000 mg/day and an adult of 70 kg has approximately 15 g of HA in the body, of which about half is found in the skin. Available data suggest that consumption of HA does not harm reproduction and development or induce cancer or allergies.

It is uncertain how much of orally administered HA is absorbed from the intestine of humans after supplement intake. In animal studies, the amount of absorbed HA varies from almost no absorption to about 90%, depending on the study design and molecular weight of the HA tested. Smaller fragments of HA can be absorbed unaltered, while HA of high molecular weight seems to be degraded into smaller fragments by intestinal microorganisms before uptake, and further degraded into monosaccharides in the liver. In some studies, around 75-90% of the orally administered HA is catabolised and the degradation products are excreted in urine or expired as CO_2.

No adverse health effects of HA intake from dietary supplements have been reported based on information from 17 randomized controlled trials (RCTs) with a total of around 1000 participants, of which around 500 have received HA doses from 25 to 225 mg/day. The longest study lasted 12 months, with a dose of 200 mg per day. The RCTs were mainly designed to investigate potential beneficial effects of HA treatment. Safety was assessed and documented to varying degrees. Several studies collected blood and/or urine to investigate changes in hematology, liver- or kidney function after HA intake, but the results were only shown in four studies that included around 130 participants receiving HA.

No sub-chronic toxicity has been reported following oral administration of HA in eight experimental animal studies in rats lasting for 90 days. All 90-day studies reported body and organ weight, histopathology, haematology, and blood biochemistry. The highest dose given (two studies) was 1333 mg/kg body weight and did not lead to negative health effects. This dose was used as a reference point in the hazard characterization.

The reference point of 1333 mg/kg body weight from animal studies was compared to HA exposure in humans of 2.9 mg/kg body weight per day, corresponding to a daily supplement dose of 150 mg HA for an adult weighing 52 kg. The margin of exposure of 460 (ratio 1333/2.9) is considered sufficiently large to suggest that a HA exposure of 2.9 mg/kg body weight and lower (reflecting HA doses 150 mg/day and lower), are not associated with known health risks. Because the RCT studies of health effects of HA supplementation lasted up to 12 months and had relatively few participants, VKM cannot conclude on effects over a longer period, or rare side effects.

Key words: Hyaluronan, hyaluronic acid, sodium hyaluronate, dietary supplements, adults, adverse health effects, toxicology, risk assessment, "other substances", VKM, Norwegian Scientific Committee for Food and Environment, Norwegian Food Safety Authority

Sammendrag på norsk

Hyaluronsyre (HA) er et polysakkarid med høy molekylmasse som produseres i kroppen og fins i bindevev, leddvæske, øyevæske og hud. Grunnenheten i HA polymeren består av D-glukuronsyre og N-acetyl-D-glukosamin.

Mattilsynet har bedt VKM om å vurdere risikoen for skadelige helseeffekter knyttet til daglig inntak av kosttilskudd med 150 mg, 120 mg, 64 mg, og 48 mg HA. VKM har ikke beregnet bidrag fra andre kilder til HA (mat, kosmetikk og ulike medisinske anvendelser).

HA for konsum utvinnes fra hanekam og kyllingbrusk eller produseres ved bakteriell fermentering. Hanekamekstrakt ble vurdert av den europeiske myndighet for næringsmiddeltrygghet (EFSA) som ingrediens i «ny mat» i 2013. Et daglig inntak av 80 mg hanekamekstrakt med et innhold på 60 – 80 prosent HA ble funnet trygt. Dette tilsvarer 48 - 64 mg HA per dag.

Kroppens egen produksjon av HA er rundt 5000 mg/dag. En voksen person på 70 kg har ca. 15 g HA i kroppen, hvorav omtrent halvparten finnes i huden. Tilgjengelige data indikerer at inntak av HA ikke skader reproduksjon og utvikling, eller bidrar til kreft og allergi.

Det er usikkert hvor mye HA som tas opp fra tarm hos mennesker etter inntak av kosttilskudd. I dyrestudier hvor HA er gitt oralt, er det rapportert om nesten ingen absorpsjon til ca. 90 prosent absorpsjon, avhengig av studiedesign og molekylvekt. Mindre fragmenter av HA kan absorberes uendret, mens HA med høy molekylvekt ser ut til å brytes ned av mikroorganismer i tarmen før opptak for deretter å brytes videre ned i leveren til monosakkarider. I noen studier blir rundt 75 – 90 prosent av oralt administrert HA brutt ned (katabolisert) og restprodukter blir skilt ut i urinen eller som CO_2.

Det er ikke rapportert om skadelige helseeffekter av HA fra kosttilskudd. Dette er basert på informasjon fra 17 randomiserte kontrollerte studier (RCTer) med totalt rundt 1000 deltagere, hvorav rundt 500 har fått HA i doser fra 25 til 225 mg/dag. Den lengste studien varte i 12 måneder, her var dosen 200 mg per dag. De randomiserte kontrollerte studiene undersøkte hovedsakelig nytten av HA-tilskudd. Mulige negative helseeffekter ble undersøkt og dokumentert i varierende grad. Flere studier samlet inn blod og/eller urin for å undersøke endringer i hematologi, lever- eller nyrefunksjon etter HA-tilskudd, men resultatene ble kun vist i fire studier som omfattet rundt 130 deltakere som mottok HA.

Det er ikke rapportert om subkronisk toksisitet etter oral tilførsel av HA i åtte eksperimentelle studier i rotter med varighet på 90 dager. Alle studiene vurderte og rapporterte kropps- og organvekt, histopatologi, hematologi og blodbiokjemi. Den høyeste dosen som ble gitt (to studier) var 1333 mg/kg kroppsvekt og førte ikke til negative helseeffekter. Denne dosen ble brukt som referansepunkt i farekarakteriseringen.

Referansepunktet 1333 mg/kg kroppsvekt for HA fra dyrestudier ble sammenlignet med HA eksponering i mennesker på 2,9 mg/kg kroppsvekt per dag. Dette tilsvarer en daglig dose kosttilskudd med 150 mg HA for en voksen person som veier 52 kg. Eksponeringsmarginen på 460 (ratio 1333/2,9) anses som tilstrekkelig stor til å antyde HA-eksponering på 2,9 mg/kg kroppsvekt og lavere (som gjenspeiler HA-doser 150 mg/dag og lavere), vurderes til ikke å være forbundet med kjent helserisiko. Fordi RCT studiene av helseeffekter av HA fra kosttilskudd varte opp til 12 måneder og hadde relativt få deltagere, kan ikke VKM konkludere på effekter over lengre tid, eller sjeldne bivirkninger.

Abbreviations and glossary

Abbreviation

ADME	absorption, distribution, metabolism, and excretion
bw	body weight
Da	Dalton
EC	European Commission
EEA	European Economic Area
EFSA	European Food Safety Authority
FDA	United States Food and Drug Administration
GRAS	Generally Recognized as Safe
GRN	GRAS Number
HA	hyaluronic acid
kDa	kilo Dalton
LOAEL	lowest observed adverse effect level
MDa	Mega Dalton
MOE	margin of exposure
MW	molecular weight
NaHA	sodium hyaluronate
NFSA	Norwegian Food Safety Authority
NOAEL	no observed adverse effect level
RCE	rooster comb extract
RCT	randomized controlled trial
VKM	Norwegian Scientific Committee for Food and Environment

Glossary

Absorption, distribution, metabolism, and excretion (ADME)

The four key processes which describe how drugs and chemicals get into the body, what happens to them while they are there, and how they are eliminated.

Adverse health 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).

Lowest observed adverse effect level (LOAEL)

The lowest level of a substance that has been observed to cause harm in an exposed population (EFSA glossary)

Margin of exposure (MOE)

The gap, expressed as a ratio, between the actual intake (exposure) to a potential toxic substance in a population and a value (e.g. Reference Point, RP or Point of Departure, POD) characterizing its hazard. MOE =RP/Exposure

No observed adverse effect Level (NOAEL)

The greatest concentration or amount of a substance at which no detectable adverse effects occur in an exposed population (EFSA glossary).

Oral toxicity

Toxicity by the oral route of exposure.

"Other substances"

A substance other than a vitamin or mineral that have a nutritional or physiological effect (Regulation (EC) No 1925/2006 of the European Parliament and of the Council).

"Positive list"

Annex to Regulation (EC) No 1925/2006 including "other substances" and levels thereof allowed for addition to foods.

Background as provided by the Norwegian Food Safety Authority

"Other substances" are substances that have a nutritional or physiological effect and are not vitamins or minerals. Examples of "other substances" include fatty acids, amino acids, coenzyme Q10 and caffeine. Excessive intake of certain "other substances" may be associated with health risks.

In the European Economic Area (EEA), the provisions on the addition of "other substances" to foods are currently only partially harmonised in Regulation (EC) No 1925/2006. This means that Member States may lay down national supplementary provisions on the aspects that are not harmonised. Any national supplementary provisions must comply, inter alia, with the general principles of EEA law on the free movement of goods, the principles of mutual recognition and the exemptions from these EEA legal principles.

In Norway new supplementary national provisions regarding the addition of certain "other substances" to foods including food supplements entered into force on 1 January 2020. These provisions are included in the Norwegian regulation on the addition of vitamins, minerals and certain other substances to foods (in Norwegian: "Forskrift om tilsetning av vitaminer, mineraler og visse andre stoffer til næringsmidler"), which also implements Regulation (EC) No 1925/2006 in Norwegian law.

A so-called "positive list" for the addition of certain "other substances", was introduced as Annex 3 to the regulation. The intention of the Norwegian supplementary provisions is to reduce health risks that can occur when consuming certain "other substances" in foods, including food supplements.

The Norwegian supplementary provisions only apply (Section 6, second paragraph) to the addition of "other substances" that a) have a purity of at least 50% or are concentrated 40 times or more, and b) are not normally consumed as a food in themselves and not normally used as an ingredient in foods. Furthermore, the supplementary national provisions do not apply (Section 6, third paragraph) to the addition of the following "other substances": a) plants or parts of plants in fresh, dried, chopped, cut, or powdered form, b) extracts of plants or parts of plants exclusively made through basic aqueous extraction, possibly followed by dehydration, c) enzymes and microorganisms and d) "other substances" listed in Parts A and B of Annex III to Regulation (EC) No 1925/2006.

It is only permitted to add "other substances" that are listed in the "positive list" in Annex 3 to foods, including food supplements. The addition must be in accordance with the terms and conditions set in the "positive list", including the threshold values that are set for the different substances.

If a food business operator wants to add a higher quantity of a substance or add a substance that is included in the "positive list" to a new category of food products, the food business operator must notify the Norwegian Food Safety Authority (NFSA, see Section 9). If a food business operator wants to add new substances, not currently included in the "positive list", the food business operator must apply for authorization to NFSA (see Section 10).

For NFSA to processes an application or notification, NFSA may request that the Norwegian Scientific Committee for Food and Environment (VKM) performs a risk assessment of higher amounts of substances listed in the "positive list", or new substances with applications for authorization.

Terms of reference as provided by the Norwegian Food Safety Authority

The NFSA asks the Norwegian Scientific Committee for Food and Environment (VKM) to assess whether HA (CAS number 9067-32-7) in food supplements intended for adults aged 18 years and older may pose a health risk in the Norwegian population.

The NFSA asks VKM to consider daily intake of food supplements with 150 mg, 120 mg, 64 mg, and 48 mg hyaluronic acid, respectively.

This includes:

• Identify and characterise adverse health effects.

- ✓ Identify harmful health effects and describe at what doses these occur.
- ✓ Describe uncertainty related to knowledge about health effects and dose and in case of possible extrapolation from animals to humans.

• Evaluate the exposure.

- ✓ Evaluate exposure for the dose(s) and age groups given above.
- ✓ Describe uncertainty related to the exposure evaluations.

• Characterise health risks associated with exposure to hyaluronic acid and describe uncertainty that may have an impact on the conclusions.

• Identify and describe knowledge gaps that may have an impact on the conclusions.

Assessment

1 Introduction

Hyaluronic acid (HA) is a high-molecular-mass polysaccharide endogenously produced and present in connective tissue in all vertebrates and some microalgae. Total exposure includes intake of animal foods or ingredients based on animal parts (rooster comb, connective tissue), and use of dermal cosmetic products and medical products. On the European market, medical products containing HA exist for the treatment of wounds, as surgical aid in ophthalmology, and for injections into joints for management of osteoarthritis symptoms. HA is also used as a dietary supplement. Common indications for supplement use are osteoarthritis in knees and other joints, and skin conditions, such as dry skin and wrinkles.

Traditionally, HA for use in pharmaceuticals and foods is extracted from rooster combs or chicken sternal cartilage but is now mainly produced via bacterial (streptococcal) fermentation (Liu et al., 2001). Molecular weight (MW) is an important specification of a commercial HA product (Armstrong and Johns, 1997) as it determines the physiological response and properties such as viscoelasticity, moisture retention and mucoadhesion, that help to define the appropriate applications. VKM has been requested to evaluate supplements that contain HA in the form of rooster comb extract (RCE), or HA produced by bacterial fermentation. In these supplements, the MWs range from 800 kilodaltons (kDa) for HA from rooster comb to 0.2-0.6 megadaltons (MDa) for HA from fermentation.

Commercial HA powder has been approved as a food ingredient outside Europe, and as a novel food ingredient in Europe. In 2013, the European Food Safety Authority (EFSA) adopted a Scientific Opinion that concluded that RCE with a HA content of 60-80% is safe under the proposed use in dairy products at a maximum dose of 80 mg per portion and per day, corresponding to 48-64 mg HA per day (EFSA 2013). Based on this opinion the DTU National Food Institute in Denmark ("Fødevareinstituttet") has concluded that daily intake of HA through food supplements at a dose of 48 mg/day for HA derived from RCE (Poulsen, 2019), 50 mg/day for HA derived by bacterial fermentation (Poulsen, 2020) will not lead to any adverse health effects. A maximum dose of 64 mg/day for HA from RCE in dietary supplements has later been implemented in Danish regulation. VKM has been requested by the NFSA to assess the safety of intake of HA from food supplements at doses of 48 mg/day and 64 mg/day, reflecting the interval in the EFSA opinion from 2013 and Danish regulation, as well as two higher daily doses (120 mg and 150 mg) in applications to the NFSA.

1.1 Delimitations

- The risk assessment is performed for oral intake of HA as a food supplement and only for the doses in the mandate given by NFSA.
- Intake of HA from foods or exposure from other sources (cosmetic- and medical use) is not estimated by VKM.
- The age group included (adults 18 years and older) is given in the mandate from the NFSA.
- Documentation of any beneficial effects of HA is not evaluated.
- Interactions between HA and other components is not addressed.
- Stability of HA in dietary supplements is not addressed.

2 Substance specifications

Hyaluronic acid is (HA) a non-sulphated glucosaminoglycan. Sodium hyaluronate hyaluronic acid sodium salt, hyaluronan), CAS-number 9067-32-7, is the sodium salt of HA (NaHA). HA is a polymer of a basic unit consisting of D-glucuronic acid and N-acetyl-D-glucosamine linked by a ß-1,3-glycosidic bond that are polymerized via ß-1,4-glycosidic bonds (Figure 2-1). The molecular formula and mass of the HA sodium salt are $(C_{14}H_{20}NO_{11}Na)_n$ and $(401.34)_n$.

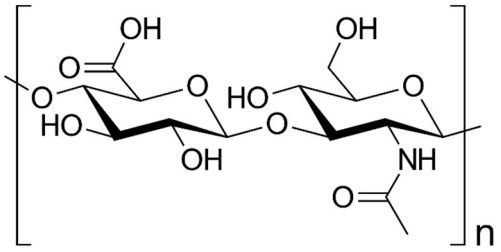

Figure 2-1 Structure of hyaluronic acid showing the basic repeating unit of hyaluronic acid polymer.

Natural HA in the human body occurs mainly as salts and the MW is around 10^3–10^4 kDa. Sodium hyaluronate is mainly produced from rooster or chicken comb extracts or from fermentation of microorganisms. In commercial products, the MW of sodium hyaluronate can vary considerable from very low (<2 to 10 kDa) to very high (1.8 to 2.2 MDa). When HA is derived from an animal source, proteins may be present, which can affect the nonimmunogenic and immunogenic properties of the hyaluronic acid preparation.

Name and other identifiers of sodium hyaluronate are presented in Table 2-1 (Source: PubChem database from the U.S. Institute of National Health: https://pubchem.ncbi.nlm.nih.gov/compound/3084049)

Table 2-1 Name and other identifiers of sodium hyaluronate.

Substance name	Sodium hyaluronate
Synonym	• Hyaluronic acid • Hyaluronic acid, sodium salt • Hyaluronan (Medical Subject Heading, MeSH)
CAS number	9067-32-7
European Community (EC) Number	618-620-0
PubChem CID	3084049

"HA" is used to describe hyaluronic acid or sodium hyaluronate in many literature references. Sometimes it is not clear which form is being discussed. VKM uses "NaHA" when it is clear that the sodium form is being discussed. "HA" is used for the acid form or when the form is unclear, and when referenced articles use "HA."

3 Exposure

3.1 Doses as specified by the Norwegian Food Safety Authority

VKM has evaluated the daily intake of food supplement doses of 150 mg, 120 mg, 64 mg, and 48 mg HA, as requested by the NFSA. Exposure from other sources of HA is not estimated by VKM.

Default body weights (bw) determined by EFSA (2012) for the EU adult population, were used to estimate the daily intake of HA in the unit mg/kg bw. Intake was estimated for the 5th percentile (P5) and the 50th percentile/median (P50) of bw for men and women combined (Table 3.1-1).

Table 3.1-1 Estimated daily exposure to hyaluronic acid per kg body weight for the supplement doses given by the Norwegian Food Safety Authority.

		Body weight (bw)		Daily intake	
Age group	**Daily supplement dose**	**P5**	**P50**	**Individuals with P5 bw**	**Individuals with P50 bw**
Adults ≥18 years	150 mg	52 kg	72 kg	2.9 mg/kg/day	2.1 mg/kg/day
	120 mg	52 kg	72 kg	2.3 mg/kg/day	1.7 mg/kg/day
	64 mg	52 kg	72 kg	1.2 mg/kg/day	0.9 mg/kg/day
	48 mg	52 kg	72 kg	0.9 mg/kg/day	0.7 mg/kg/day

P5=5th percentile, P50=50th percentile (median)

3.2 Endogenous production

HA is synthesized in the plasma membrane by membrane bound hyaluronan synthases in humans. It is especially abundant in connective tissue and present in all organs of the body. The highest HA concentrations is found in the umbilical cord, in the cartilage tissue, in the vitreous humour of the eye, in synovial fluid and skin. In an individual of 70 kg there are about 15 grams of HA of which about half is found in the skin (Fraser et al., 1997; Becker et al., 2009). About 20-30% of the turnover in skin and skeletal tissue occurs locally whereas the rest is cleared by the lymphatics (Fraser et al., 1997). The tissue half-life is estimated to be less than one day to three days. About 5000 mg HA is recycled daily, i.e. catabolised into small oligomers, sugars, glucuronic acid and glucosamine, and entering other metabolic routes (Stern 2004), indicating resynthesis and dietary intake of a similar amount of HA.

The production of HA decreases with age (Fraser et al., 1997). The concentration of HA in plasma of healthy humans have been reported to be in the range of 10-100 ng/ml with a mean value of 30-40 ng/ml (Lebel 1991).

3.3 Other sources

HA is a common natural component of food. It is present in animal products where cartilage and bone are present and would be extracted when present in soups or stews. Rooster comb is particularly high in HA and therefore used as raw material for some commercial HA products. VKM has not been able to find numerical estimates of background exposure to HA from the diet or the concentration of HA in foods other than rooster comb. One manufacturing company (Bioibérica, Spain) has reported in their novel food application to EFSA that their HA extraction process from rooster comb yields 1% extract. A rooster comb of approximately 8 grams will give 80 mg RCE, containing 60-80% HA (EFSA 2013). A call for food composition data by VKM (March 10, 2023) through the FAO/INFOODS network (International Network of Food Data Systems), did not generate any information on HA in foods before the publication of the current report.

HA has a wide range of cosmetic and medical applications. HA penetrates the skin and has been considered a safe cosmetics ingredient in a review by the Cosmetics Ingredient Review program (Becker et al., 2009). At the request of the U.S. Food and Drug Administration (FDA), ECRI (ecri.org) has reviewed research and developed safety profiles for HA as medical device for muscle/skeletal, dermal/facial/eye applications, and adhesion barrier and bulking agent applications (ECRI 2021). Examples of these applications are eye gels, oral gels, and dermal injections or into joints for conditions such as osteoarthritis. Medical use of HA is probably very limited in Norway. There are currently no medicines with market authorisation. Prescribers may apply for compassionate use, named patient (in Norwegian: Godkjenningsfritak) for two products only: one injection solution for intravesicular (bladder) use (Cystistat injection containing 40 mg HA per/50 ml) and one eye gel (Vismed containing HA at 0.3% weight per volume, w/v) (Source: correspondence with the Norwegian Medicines Agency). The products are registered in the Norwegian Pharmaceutical Product Compendium (in Norwegian: Felleskatalogen) which is available online (https://www.felleskatalogen.no/). VKM has not searched for estimates of exposure to HA from cosmetics use such as dermal fillers, which are not regulated as a medical product, or from medical products distributed without prescriptions (over-the-counter).

4 Hazard identification and characterisation

The questions for the hazard identification and characterisation for oral intake of HA hyaluronic acid (HA) are presented in Table 4-1. Potential negative effects were divided into genotoxicity, reproductive toxicity, and other adverse effects (referred to as adverse health effects), including allergenicity.

Table 4-1 Hazard questions for hyaluronic acid (HA).

Absorption, distribution, metabolism, and excretion (ADME)	1	What is the ADME of HA in humans? Is human and animal (rodent) ADME similar?
	2	Is HA metabolised to innocuous metabolites?
	3	Is HA endogenous to humans? If yes, is the dose given in the mandate from NFSA resulting in body levels within the range normally metabolized and eliminated?
Hazard identification	4	Is there a concern for genotoxicity or reproductive toxicity?
	5	Is exposure to HA associated with adverse health effects?
Hazard characterisation	6	Is there a dose-dependent relationship between exposure to HA and the adverse effects?

To identify relevant data to answer the research questions in Table 4-1, several information sources were searched:

- Previous opinions, risk- or safety assessments of HA found through the websites of the following international risk assessment and medicine evaluating organisations and agencies: the U.S. Food and Drug Administration (FDA), the US National Academies of Sciences, Engineering, and Medicine (NASEM), the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the Norwegian Medicines Agency, and the European Chemicals Agency (ECHA).
- A search for scientific literature on oral intake of HA was conducted by VKM to identify animal toxicology studies and RCTs in humans that have assessed potential adverse health effects.
- Documentation provided by the NFSA. Confidential information supplied by one applicant was used in assessing the allergenicity of rooster comb extract.

The following opinions were considered relevant and are summarized below:

Conclusion of GRAS Status of Sodium Hyaluronate. Food Usage Conditions for General Recognition of Safety. GRAS Notice (GRN) No. 976 to US Food and Drug Administration from 2020

GRAS (Generally Recognized As Safe) is a regulatory designation set by the U.S. FDA as defined by the Federal Food, Drug and Cosmetic Act. GRAS status applies to any substance intentionally added to a food, that is not a food additive, colour additive, or prior sanctioned ingredient. GRAS Associates, LLC (a Limited Liability Company) offers food safety regulatory services for U.S. market access. At the request of Bloomage Biotechnology Corporation, Ltd., GRAS Associates has determined that their sodium hyaluronate (NaHA) of MW range 10-4000 kDa that is produced by microbial fermentation is GRAS, under the intended conditions of use, i.e. as an ingredient in beverages, including fruit juices and carbonated soft drinks, candy, milk and milk products, and ready-to-eat cereals. The maximum amount per serving ranged from 40-60 mg NaHA, depending on food group. Bloomage estimated the mean and 90^{th} percentile dietary intakes of NaHA to be 125 mg/day and 250 mg/day, respectively, for the proposed intended use (GRAS Assoc. 2020). VKM noted that GRAS Associates were not able to find data on background exposure to HA from dietary intake (GRAS Assoc. 2020).

The report from GRAS Associates (2020) includes a non-systematic but comprehensive review of animal studies of oral toxicity of HA. In general, many animal studies have been published in Chinese or Japanese and GRAS Associates (2020) also includes references to some Chinese studies cited as "unpublished" (from the Shandong Center for Disease Control and Prevention; the Institute for Nutrition and Food Safety, Chinese Center for Disease Control and Prevention; and Bloomage Biotechnology Corp.). VKM has to a large extent relied on the GRAS report and other reviews with English summaries to include as much evidence as possible from animal studies without restrictions on language, or publication status (published or "unpublished").

Scientific Opinion on Rooster Combs Extract (EFSA 2013)

The EFSA Panel on Dietetic Products, Nutrition and Allergies assessed the safety of RCE as a novel food ingredient following an application by Bioibérica to put RCE on the market. Following standard procedures for applications for novel foods, EFSA concluded that RCE is safe under the proposed use. The applicant intended to add RCE to liquid milk, milk-based products (fermented beverages), yoghurts and fromage frais with a recommended maximum intake of 80 mg RCE per portion and per day where HA is about 60-80% of the extract, i.e 48-64 mg HA. The target population was the general population, except for pregnant women, children, and people with adverse reactions to sodium hyaluronate and/or avian protein (EFSA 2013).

VKM has noted that the supporting literature in the EFSA 2013 opinion was limited to one study on genotoxicity, two animal toxicity studies in rats (one on high dose acute toxicity and one on subchronic 90-day oral toxicity), and two human RCTs.

Cosmetic Ingredient Review: Final Report of the Safety Assessment of Hyaluronic Acid, Potassium Hyaluronate, and Sodium Hyaluronate

The Cosmetic Ingredient Review program, a non-profit program to assess the safety of ingredients in personal care products, reviewed the safety of HA as ingredient in cosmetic formulations (Becker et al., 2009). The review combines published studies and unpublished data provided by interested parties to the program, to assess the safety including the potential for genotoxicity and carcinogenicity, reproductive and developmental toxicity, and immunogenicity. This report concludes that HA was not toxic in a wide range of acute animal toxicity studies, over several species and with different exposure routes, but with limited evidence on oral toxicity.

4.1 Absorption, distribution, metabolism, and excretion (ADME)

4.1.1 Absorption

Studies on absorption, distribution and excretion have been conducted in experimental animals using various HA products and techniques. The absorption of HA is unclear and apparently conflicting results have been reported (Zheng et al., 2023).

Artificial gastric juice and intestinal juice seems unable to degrade HA, whereas orally administered HA can be degraded by caecal microorganisms (Kimura et al., 2016).

In studies with human intestinal Caco-2 cells, low MW HA <5 kDa could pass paracellular pathway more easily than HA of up to 70 kDa, while HA above 100 kDa did not pass (Hisada et al., 2008).

The absorption of ^{99m}Technetium labelled high MW HA products (MW about 1-1.5 MDa) was investigated in rats and beagle dogs (Balogh et al., 2008). It was found that in rats, 85 to 92.3% was excreted in faeces for 72 hours, whereas only 2-3.2% was found in urine. In beagle dogs, only trace amounts were seen in blood after oral administration of the same products. Small amounts, up to a few percent of the dose of radioactivity were also found in bone, joints, muscle, and skin tissues after a few hours. A completely different pattern of absorption and distribution were seen with ^{99m}Technetium pertechnetate as a control.

The absorption of ^{99m}Technetium labelled HA of different MW, 0.1-1 MDa was investigated in rats (Laznicek et al., 2012). No significant absorption and only traces were detected in blood following oral administration regardless of MW.

In a study by Oe and co-workers (Oe et al., 2014) ^{14}C-labelled HA with an average MW of 920 kDa was given orally (25 mg/kg bw) or intravenously (10 mg/kg bw) to rats. After oral administration, radioactivity in plasma was observed after 4 hours, reaching a peak at 8 hours. The absorption was around 90%, but the bioavailability in the central compartments was calculated to 16% using the area under the curve of HA in plasma.

In a study in rats, transfer of orally administered HA to blood and lymph was investigated (Sato et al., 2020). The rats were given HA with average MW of 2, 8, 50 and 300 kDa. HA concentrations were determined using a commercial ELISA kit for HA that according to the manufacturer is minimally affected by molecular size of HA, with quantification of any HA above 7.4 kDa. Peripheral plasma concentrations were monitored for 24 hours after administration. Only for HA of 2 and 8 kDa an increase in plasma was observed. In the portal vein plasma concentration following 2 kDa HA administration increased up to 2 hours. Samples were also obtained from ductus thoracicus during 24 hours showing increased concentrations of 2 kDa HA from 2-24 hours, whereas only trace amounts of 8 kDa HA were observed after 6-8 hours. No absorption was observed for 50 and 300 kDa HA indicating that these are not absorbed intact.

4.1.2 Distribution

Following intestinal absorption, HA is distributed to various parts of the body via blood circulation and the lymphatic system. It may both be transported directly via lymphatic transport to peripheral tissues or via the liver where further metabolization may take place. Cellular uptake depends on HA concentration and MW. HA and HA fragments are taken up in cells by endocytosis subsequent to receptor binding at the cell surface (Zheng et al., 2023). Following oral administration of 25 mg/kg bw of ^{14}C-labelled HA with an average molecular weight of 920 kDa to rats the elimination half-life in plasma was 1.9 days, while after intravenous administration the elimination was biphasic with a half-life of 1.3 hours during the first 8 hours and 1.7 days during 24 to 168 hours (Oe et al., 2014). The tissue distribution after oral administration was studied by autoradiography. While the main part remained in the gastrointestinal tract, radioactivity distributed to most organs and reached a maximum level after 8 hours and disappeared after 2 days. The chemical nature of the radioactivity is not known but is likely to be HA breakdown products as other evidence indicate no or very little absorption of intact HA with a molecular weight above 50 kDa (Sato et al., 2020). Intravenously given ^{99m}Technetium-labelled HAs of different molecular weights were for a large part found in the liver, spleen, and kidney (Laznicek et al., 2012). In rats and dogs given ^{99m}Technetium-labelled HA of high molecular weight, radioactivity detected by scintigraphy was located to joints, vertebra, and skin tissues, in particular (Balogh et al., 2008).

4.1.3 Metabolism

Oral HA is to a large extent metabolised by intestinal bacteria in the lower part of the intestinal tract where it is degraded into small fragments by microbial hyaluronidases. In the liver and other tissues hyaluronidases may further degrade HA. Degradation into monosaccharides takes place by glucosidases in the liver where the monosaccharides may be used for synthesis or enter energy metabolism and conversion into CO_2. It was found that around 75% of orally administered ^{14}C-HA of 920 kDa to rats was catabolised to CO_2 and expired (Oe et al., 2014).

In the tissues turnover and catabolism of HA, mediated mostly by hyaluronidases, occur locally subsequent to endocytosis of cells in extracellular matrix, followed by clearance to the lymphatic system and further degradation in lymph nodes, spleen, liver and kidney (Zheng et al., 2023).

Complex systems of several enzymes and receptors operates in HA catabolism and HA synthesis, which are strictly regulated to balance and maintain tissue homeostasis and avoid excessive accumulation of HA (Zheng et al., 2023).

Both HA and its catabolites are found naturally in the body. The metabolites of HA can therefore be considered innocuous in physiological amounts.

4.1.4 Excretion

Unabsorbed HA is excreted in faeces. HA degradation products are excreted in urine and HA degraded into volatile CO_2 is exhaled.

4.1.5 Summary ADME

In summary, conflicting results are reported for the level of absorption of oral HA, ranging from a few percent to 90%, and excretion levels above 90% are accordingly reported. It is possible that only intact HA of low molecular weight (< 2 kDa) can be absorbed and to a low extent, however this remains uncertain. Absorption routes are both via portal circulation to the liver and via the lymphatic system directly to the systemic circulation. HA degradation products seemingly produced by the intestinal microbiota, but not characterized, may be absorbed, and distributed to various parts of the body. These metabolites enter normal metabolism where they are further processed as building blocks in synthetic pathways or further catabolised to CO_2.

4.2 Genotoxic potential and carcinogenicity

Gene mutations and structural and numerical chromosomal alterations are the endpoints that should be addressed to evaluate genotoxic potential.

The genotoxic potential of hyaluronic and RCE has been reviewed in previous safety assessments identified by VKM. The review by GRAS Associates (2020) is by far the most comprehensive regarding the number of studies and range of assays and covers primary studies in other reviews (Becker et al., 2009; EFSA 2013; Oe et al., 2016). Negative results have been reported using bacterial reverse mutation assays, mouse micronucleus testing, mouse sperm malformation testing (up to 5000 mg NaHa/kg bw), and chromosomal aberration assays (GRAS Assoc. 2020). EFSA had no safety concerns related to the genotoxicity of RCE which has been tested using a bacterial reverse mutation assay (EFSA 2013).

Elevated levels of often occur in the surrounding stroma of malignant tumours and have been associated with tumour progression and cancer cell migration (Hill et al., 2006; Sironen et al., 2011; Sato et al., 2016). HA expression in tumour cells or surrounding stroma is associated with poor survival in patients with gastric and colorectal cancers, prostate, breast- and ovarian cancers (Sato et al., 2016). GRAS Associates (2020) noted that "Despite the apparent role of endogenous HA dysregulation in cancer progression, little is known about the carcinogenicity of increased systemic levels of HA resulting from exogenously administered HA (*i.e.*, oral, or subcutaneous). The Cosmetic Ingredient Review Expert Panel concluded in 2009 that HA is not thought to have a causal role in tumour metastasis. Rather increased expression of HA genes may be a consequence of metastatic growth and give tumour resistance to chemotherapeutic agents (Hill et al., 2006; Becker et al., 2009; Sato et al., 2016).

In summary, VKM concludes that available data suggests no genotoxic potential or carcinogenicity for exogenous HA administration.

4.3 Reproduction toxicity

GRAS Associates (2020) did not review reproductive and development studies but refers to a comprehensive review of the reproductive toxicity of NaHA ('Hyaluronate (CAS 9067-32-7) REPROTOX Record Number 4179' from REPROTOX.org) concluding that HA is a naturally occurring glycosaminoglycan not expected to adversely affect pregnancy outcomes. This conclusion was based on no adverse outcomes in rat and rabbit fertility and pregnancy studies at doses ranging from 40-64 mg/kg bw/day. The highest dose of NaHA tested in a teratogenicity study in rat, revealed a NOAEL of 1333 mg/kg bw/day (cited in GRAS Assoc. 2020: Peng et al. (2011), in Chinese).

4.4 Allergenicity

As a carbohydrate, HA is not expected to exhibit any allergenic activity. However, hyaluronic acid derived from animal sources, such as rooster comb, can contain proteins or other impurities that may cause allergic responses. The Panel in EFSA (2013) considered that the risk of allergic reactions to RCE is not dissimilar to other products derived from chicken meat. GRAS Associates (2020) found no reports of allergy from oral consumption of HA in the literature. In confidential technical information submitted to the NFSA for a product based on RCE, tests by the applicant showed no cross reactivity with egg proteins known to elicit allergic reactions. Hydrolysed proteins from the product did not bind serum IgE from egg allergic patients. Two animal studies of antigenicity and systemic anaphylactic reactions after NaHA injections (intraperitoneal or intramuscular) are also reported to be negative (cited in Oe et al., 2016: Kameji et al., 1991; Takemoto et al., 1992, both in Japanese).

4.5 Adverse health effects

4.5.1 Literature search

A two-step literature search for review studies (step one) and primary research (step two) was conducted by VKM to identify previous research on the safety and potential adverse health effects of HA after oral administration in animals (usually by gavage/tube feeding) or supplement intake in humans. Both searches were conducted in January 2023 (see details below). A broad search for review studies was conducted to identity animal toxicology studies and/or human studies. Because most reviews did not present much detail on the safety assessment in human studies, it was decided to do a second search for randomized controlled trials (RCTs) in humans. This was to identify any recent primary studies and to access information on methods and results that was not reported for the studies when summarized in previous reviews.

VKM has found that much of the evidence from animal toxicology studies has been published in Chinese or Japanese, and that some human RCTs have been published in Japanese. For animal toxicology studies, VKM has therefore relied on identified reviews (Oe et al., 2016) and risk assessments (GRAS Assoc. 2020) in English that also summarise studies in Chinese and Japanese, including publications cited as "unpublished" by GRAS Associates (e.g. reports from Chinese health authorities, and the Chinese HA manufacturer Bloomage Biotechnology Corp., Ltd.). VKM has evaluated and included human RCT studies in Japanese based on machine translation to English, but translations were limited to two studies that had safety assessment as a main objective according to the English title and abstract.

The literature searches are described in more detail below. The selection of studies is reported using PRIMSA flow diagrams (Haddaway et al., 2022).

4.5.1.1 Inclusion criteria review studies

The inclusion criteria for previous review studies are listed in Table 4.5.1.1-1.

Table 4.5.1.1-1 Overview of inclusion criteria for review studies.

Criterion	Specification
Study design	Open, not specified
Population	Human or animal (rodent or mammal)
Intervention	Oral intake (humans) or administration (animals) of hyaluronic acid or rooster/chicken comb extract, all dose ranges. Duration of exposure in prioritized order (from highest to lowest): • Chronic or repeated • Subchronic • Acute
Comparator	Placebo (human) or vehicle (animal)
Outcome	All outcomes (measured or self-reported in humans) described in relation to safety, side effects, adverse health effects, or animal toxicity
Language	Studies in English or Scandinavian
Date	No limitation (inception to search date)
Type of literature	Previous review studies, systematic or non-systematic, in peer-reviewed journals

The following review studies were excluded:

- Reviews or reports without results on safety or adverse effects from primary research.

4.5.1.2 Search strategy review studies

To identify previous reviews of human or animal studies of hyaluronic acid, a broad search strategy was used. The five databases Ovid MEDLINE, Ovid Embase, Cochrane Database of Systematic Reviews, Web of Science, and Epistemonikos were searched from inception to January 3, 2023 for review studies of hyaluronic acid without further specification. The full search strategy is provided in Appendix 1. Citation searching was done by screening the reference lists of the included review studies for other eligible reviews.

4.5.1.3 Study selection review studies

The literature search identified 1976 records from the five research databases. One member of the group screened all records by title and abstract according to the inclusion criteria. Three reviews were included (Guadagna et al., 2018; Aghamohammadi et al., 2020; Sun et al., 2022). Two additional reviews (Kawada et al., 2014; Oe et al., 2016) were found by searching the reference lists of the included reviews. Next, the five reviews were read in full text by two members of the group, and two were excluded: Guadagna et al., 2018 did not support a general statement on HA safety with results from primary studies, and Aghamohammadi et al., 2020 referenced one primary study on chicken comb extract covered in other reviews. Of the three included reviews (Kawada et al., 2014; Oe et al., 2016; Sun et al., 2022), Oe et al. (2016) and Kawada et al. (2014) summarised both animal toxicology studies and human studies. Oe et al. (2016) was complemented with Kewada et al. (2014), which reported results in more detail for the studies that were included in both reviews. Kawada et al. (2014) also referenced one animal study not found in Oe et al. (2016). The last review by Sun et al. (2022) included human RCTs and was only used for citation searching to complement VKM's own search for RCTs. The selection of studies is reported in Figure 4.5.1.3-1.

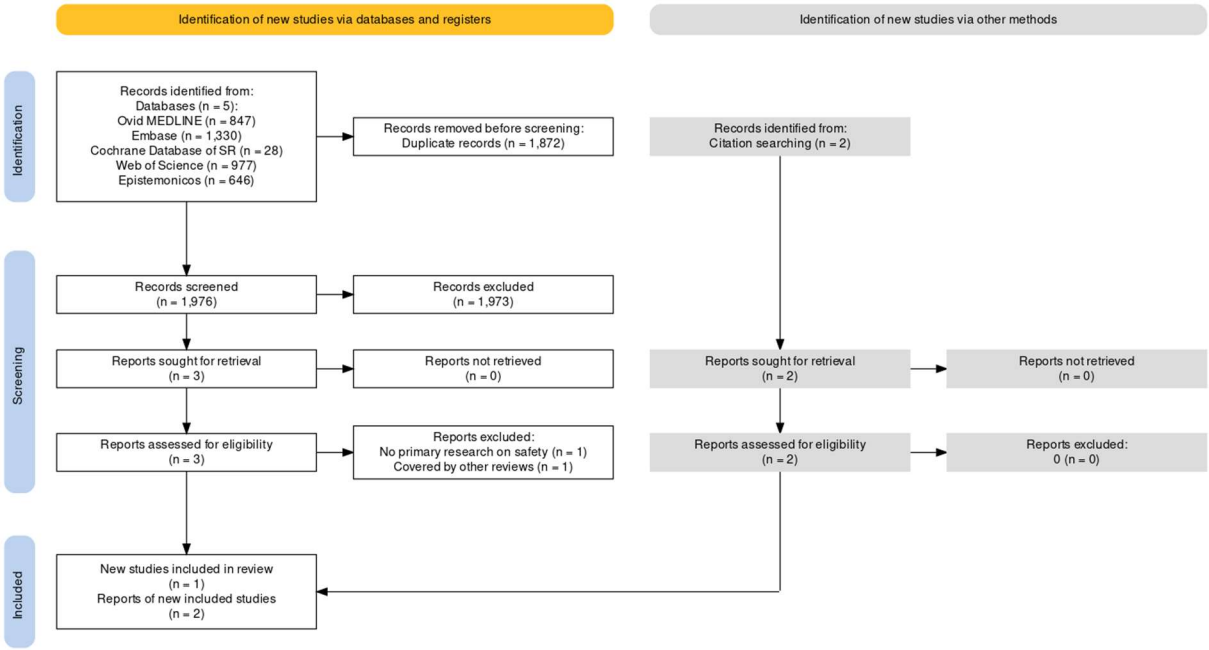

Figure 4.5.1.3-1 Flow diagram for the selection of review studies.

4.5.1.4 Internal validity/risk of bias assessment review studies

None of the included reviews qualified as systematic reviews of the safety of HA. Either no methods section or search strategy was reported, or assessment of safety/adverse effects was not the primary objective of the study. The internal validity (risk of bias) was not assessed further. VKM relied on reviews published in English for summaries of animal toxicology studies as the main body of evidence was found to be published in Chinese or Japanese. Because the research reviews were non-systematic, they were compared and supplemented with summaries in previous risk assessments for a more complete picture.

4.5.1.5 Data extraction review studies

VKM extracted data from summaries of animal toxicology studies presented in previous reviews. The data included the study ID (first author, publication year, title, language), the study design (acute, subacute, or subchronic oral toxicity), substance (hyaluronic acid, or extract from roster comb or chicken cartilage), molecular weight, the dose, dose frequency and duration of the experiment, type of experimental animal, NOAEL, and LOAEL (if highest dose tested was not equal to NOAEL), the outcomes measured, and the results.

4.5.1.6 Study inclusion criteria human RCTs

Based on the limited presentation of results from human RCTs in previous reviews, a search for primary studies was conducted to identify RCTs that addressed safety of oral intake of HA. The inclusion criteria are listed below.

Table 4.5.1.6-1 Overview of inclusion criteria for human studies.

Criterion	Specification human studies
Study design	Randomized controlled trials (RCTs)
Population	Adults
Intervention	Oral intake of hyaluronic acid or rooster/chicken comb extract, all dose ranges. Duration of intake in prioritized order (from highest to lowest): • Chronic or repeated • Subchronic • Acute
Comparator	Placebo
Outcome	All outcomes (self-report, clinical examination, or laboratory tests/biomarkers) described in relation to safety, tolerance, side effects, or potential adverse or negative health effects
Language	No restrictions. Machine translations included if safety mentioned in English title or abstract
Date	Inception to search date
Type of literature	Primary studies in peer-reviewed journals

The following exclusion criteria were applied:

- No outcomes related to safety or adverse effects
- Oral exposure from sources other than dietary supplements (e.g. mouth wash or oral gels)
- Human trials without randomization or placebo control
- HA used as addition to conventional medication in treatment of patients
- Studies without abstract in English or Scandinavian

4.5.1.7 Search strategy human RCTs

To identify RCTs, the three databases Ovid MEDLINE, Ovid Embase, and Cochrane Central Register of Controlled Trials (CENTRAL) were searched from inception to January 13, 2023 for human trials of oral intake of HA or rooster/chicken comb extract. There were no restrictions on outcomes, but included studies had to address safety, side effects, or adverse effects. The full search strategy is provided in Appendix I.

Citation searching was done by screening the result presentations in the included review studies and reports for any primary studies not identified in the search.

4.5.1.8 Study selection human RCTs

The literature search identified 1122 records from the three research databases. One member of the group screened all records by title and abstract according to the inclusion criteria. In uncertain cases, eligibility was determined by discussion with a second member. Twenty reports/publications met the inclusion criteria. In addition, seven publications were found by searching the results presentations on safety in included reviews (Kawada et al., 2014; Oe et al., 2016; Sun et al., 2022) and reports (GRAS Assoc. 2020). Next, the 27 publications were read in full text by two members of the group, and 17 were included (14 from VKM's main search and three from other reviews). The selection of studies is reported in Figure 4.5.1.8-1. Studies excluded after full-text reading (including reason for exclusion) and studies that could not be retrieved (two Japanese studies found by searching other reviews) are listed in Appendix I.

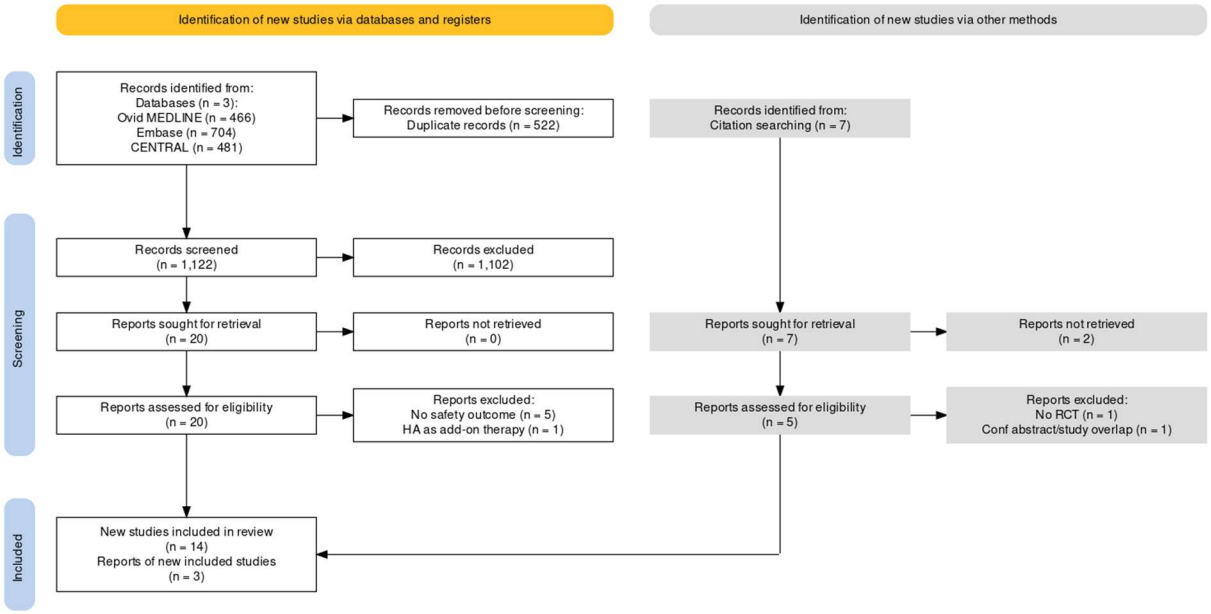

Figure 4.5.1.8-1 Flow diagram for the selection of randomized controlled trials (RCTs).

4.5.1.9 Internal validity/risk of bias assessment human RCTs

The internal validity of the human RCTs was not assessed formally. Studies with unspecified safety outcomes and/or only authors' reports of no adverse events without supporting result presentations, were judged to be of low quality/high risk of bias but were included.

4.5.1.10 Data extraction human RCTs

Data extracted from RCTs included study ID (first author, publication year, country, title), RCT design, indication for HA supplementation, study population (gender and age), study sample (numbers randomized to intervention or control group, and numbers analysed), HA product and producer, product details (source of HA, molecular weight, purity), dose given, duration of intervention, placebo used, any co-treatments (e.g. use of pain medication or physical exercise), type of safety assessment (e.g. self-reported tolerance, clinical examination, blood or urine tests) and time points, reported results, if results were supported by a presentation of results or just stated, conflict of interest (COI) statement, including corporate study funding or authorship, and trial registration number.

4.5.2 Adverse health effects (hazard identification and characterization)

4.5.2.1 Evidence description – animal toxicology studies

A total of 18 animal studies published from 1984 to 2012 were included in the identification and characterisation of potential adverse health effects of oral HA supplementation.

The following 12 studies were cited in the report by GRAS Assoc. (2020): Akasaka et al., 1988 (Japanese); Morita et al., 1991 (Japanese); Wakisaka et al., 1991 (Japanese); Ishihara et al., 1996 (Japanese); Shandong Center for Disease Control and Prevention 2004 (Chinese, "unpublished"); Institute for Nutrition and Food Safety Chinese Center for Disease Control and Prevention, 2007 and 2007b (both Chinese, "unpublished"); Schauss et al., 2007 (English); Zuo et al., 2008 (Chinese); Bioibérica 2010 (English, internal report); Canut et al., 2012 (English); Bloomage Freda Biopharm (Chinese, "unpublished", no date). The remaining six (non-overlapping) studies were cited in Oe et al., 2016: Nagano et al.,1984 (Japanese); Kato et al., 1993 (English); Tan et al., 2008 (Chinese); Yang et al., 2009 (Chinese); Guo et al., 2010 (Chinese); and Oe et al., 2011 (Japanese).

The summaries by GRAS Associates (2020) and Oe et al. (2016) together cover animal studies published in Chinese or Japanese, as well as European studies. The most comprehensive and detailed summary is found in the report by GRAS Associates (2020). Oe et al. (2016) includes additional studies in Chinese or Japanese not covered by GRAS Assoc. (2020) but provides very little detail on these studies. For some studies, additional details (origin of HA and doses) were extracted from Kawada et al. (2014). VKM did not search for primary animal studies, but Oe et al. (2016) reported one animal study (Kato et al., 1993) with an exceptionally low NOAEL compared with the other studies. Kato et al. (1993) was therefore retrieved and read in full.

Of note, Chinese and Japanese studies have with few exceptions assessed HA produced by bacterial fermentation, whereas studies from Europe have assessed HA from extracts of rooster comb or chicken sternal cartilage (GRAS Assoc. 2020).

4.5.2.2 Study characteristics – animal toxicology studies

In total of 14 of 18 studies assessed acute/subacute oral toxicity in mice, rat, or rabbit (Table 4.5.2.3-1); five assessed HA in extracts of rooster/chicken comb or chicken sternal cartilage, the remaining HA from bacterial fermentation or the origin of HA was not specified. Study duration was 14 to 30 days or was not specified. Animal numbers ranged from 20 to 40 per study (not shown) or was not specified. Single oral doses or total doses ranged from 200 to 15 000 mg/kg bw with MW ranging from 270 to 1800-2100 kDa for NaHA or was not specified. Outcomes varied by study but included examination of major organs and tissues at necropsy.

Eight studies of subchronic exposure (90 days) were all conducted in rats (Table 4.5.2.3-2).

4.5.2.3 Results – animal toxicology studies

In animal studies of acute oral toxicity, no deaths or obvious toxic symptoms were observed even at the highest dose of 15,000 mg/kg bw. According to results reported in Oe et al. (2016) and GRAS Assoc. (2020) no toxicity was observed in single-dose toxicity studies, nor repeated-dose toxicity studies (Table 4.5.2.3-1).

All 90-day studies of subchronic exposure report on body and organ weight, histopathology, haematology, and blood biochemistry with no differences found related to the exposure (Table 4.5.2.3-2). The NOAEL ranged from 12.5 to 1333 mg/kg bw. One of the first studies (Kato et al., 1993) reported the lowest NOAEL, 12.5 mg/kg bw HA, due to a significantly reduced weight gain at the exposure dose of 25 mg/kg bw. In total, 10 other studies could not verify this finding of differences in weight gain after a HA exposure between 100 to 5000 mg/kg bw. However, the 90-day study by Canut et al. (2012) also found a sporadic difference in weight gain that was not attributed to HA exposure. Small changes in the biochemical analyses were reported by Schauss et al. (2007) and Ishihara et al. (1996). However, this was not considered to be related to treatment due to the absence of a dose-related effect. This did not affect kidney or liver weight or histology and has not been reported in other studies. Based on this information, reduction in weight gain is most likely a sporadic observation not associated with exposure.

Table 4.5.2.3-1 Summary of animal safety studies on acute and subacute oral toxicity of hyaluronic acid (HA) and rooster comb extract (RCE), reported in Oe et al. (2016) and GRAS Associates (2020)

Publication	Endpoints	Origin HA	Animal	Dose mg/kg bw/day	MW	Duration	Reported results
Acute/subacute toxicity							
Morita 1991, in Japanese	Mortality & toxicological signs, body weight, macroscopic examination at necroscopy	Ferment.	Rat	200	1800-2100 kDa	14 days	LD50 > 200 mg/kg bw No signs of toxicity or deaths, no changes in bw, no macroscopic observations
Bioibérica 2010, in English	Body weight, mortality, haematology, urinanalysis, macroscopic examination	RCE	Mice	0, 5, 55, 600 RCE (60-80% NaHA) = 360-480 NaHA	800 kDa	28 days	NOAEL= 600 mg RCE/kg bw/day (360 mg NaHA/kg bw/day) No treatment-related differences in haematological or urinary analysis or weight gain
Schauss 2007, in English	Mortality & toxicological signs, body weight, macroscopic examination at necroscopy	Chicken sternal cartilage	Rats	5000 mg preparation (10% HA) = 500	NS	14 days	LD50 > 5000 mg/kg bw NaHA No deaths, normal weight gain, Macroscopic & gross pathology revealed no lesions attributable to cartilage preparation
Wakisaka 1991, in Japanese	Mortality & signs of toxicity	Ferment.	Mice	500	1800-2100 kDa	NS, single dose	LD50 > 500 mg/kg bw No mortality

Publication	Endpoints	Origin HA	Animal	Dose mg/kg bw/day	MW	Duration	Reported results
Canut 2012, in English	Mortality & toxicological signs, body weight gain, macroscopic examination at necroscopy	RCE	Rats	2000 RCE (60-80% NaHA) = 1200-1600 NaHA	800 kDa	14 days	LD50 >1200-1600 mg/kg bw NaHA No deaths, normal weight gain, macroscopic & gross pathology revealed no lesions attributable to RCE
Akasaka 1988, in Japanese	Mortality	Ferment.	Mice	1200	NS	NS, single dose	LD50 > 1200 mg NaHA/kg bw No mortality
Zuo 2008, in Chinese	Mortality & clinical signs	NS	Mice	1500	270 kDa, NS in 3 studies	14 days	LD50 >15,000 mg/kg bw No obvious toxic symptoms & no deaths
Chinese Center for Disease Control and Prevention (unpubl.), 2004	Body weight gain, food utilization, haematology, blood biochemistry Macroscopic & gross pathology of major organs & tissues	Ferment.	Rats	0, 167, 500, 1500 (NaHA)	NS	30 days	NOAL=1500 mg NaHA/kg bw, no differences found in weight gain, organ weight, biochemistry
Yang 2009, in Chinese	NS	Ferment. Calcium HA	Rats	1500	NS	30 days	NOAEL=1500 mg/kg/day bw
Chinese Center for Disease Control and Prevention (unpubl.), 2004	Mortality & toxicological signs, food consumption	Ferment.	Mice	2000 (NaHA)	NS	14 days	LD50 > 2000 mg/kg bw NaHA No obvious toxic symptoms & no deaths
Nagano 1984, 2 studies in Japanese	NS	Chicken comb	Mice Mice Rats Rabbits	NS	NS	NS, single dose	Mice: LD50 > 2400 mg/kg bw Mice: LD50 > 1200 mg/kg bw Rats: LD50 > 800 mg/kg bw Rabbits: LD50 > 1000 mg/kg bw

Publication	Endpoints	Origin HA	Animal	Dose mg/kg bw/day	MW	Duration	Reported results
Oe 2011, in Japanese	Mortality & clinical signs	NS	Rats	3500	NS	28 days	NOAEL=3500 mg/kg bw/day No toxicity was observed
Chinese Center for Disease Control and Prevention (unpubl.), 2007	Mortality & toxicological signs, food consumption	Ferment.	Rats	5280 (NaHA)	270	14 days	LD50 > 5280 mg/kg bw NaHA No obvious toxic symptoms & no deaths
Bloomage Biopharm, unpublished, in Chinese	Mortality & signs of toxicity	Ferment.	Mice	1000, 2150, 4640, 10,000 (NaHA)	NS	14 days	LD50 > 10 000 mg NaHA/kg bw No significant toxic symptoms & no deaths

BW=body weight; Ferment.=fermentation; LD50 (lethal dose 50)=amount of a substance lethal to one-half (50%) of the experimental animals exposed to it, reported as "LD50 > highest dose" when no deaths were reported; NaHA=sodium hyaluronate; NOAEL=no observed adverse effect level; MW=molecular weight; NS=not specified; RCE=rooster comb extract.

Table 4.5.2.3-2 Summary of animal safety studies on subchronic oral toxicity of hyaluronic acid (HA) and rooster comb extract (RCE), reported Oe et al. (2016) and GRAS Associates (2020)

Publication	Endpoints	Origin HA	Animal	Dose mg/kg bw/day	MW	Duration	Reported results
Subchronic toxicity							
Kato 1993, in English **Preliminary study**	Body weight gain, food consumption, organ weight, haematology, blood biochemistry, histopathology	Ferment. NaHa	Rats	3.06, 6.125, 12.5, 25, 50	870-890 kDa	90 days	NOAEL 12.5 mg/kg bw, LOAEL 25 mg/kg bw. Sporadic decrease in food consumption in 50 mg group, decreased weight gain in 25 mg group and higher, increased serum Na and Cl in 25 mg group and higher were observed in males but not in females. No differences between the groups were observed for other outcomes (more details in section 4.5.2.4)

Publication	Endpoints	Origin HA	Animal	Dose mg/kg bw/day	MW	Duration	Reported results
Ishihara 1996, in Japanese	Animal and organ weight, food efficiency and behaviour, haematology, blood biochemistry, urinalysis, histopathology	Ferment. NaHA	Rats	0, 3, 12, 48	NS	90 days	NOAEL= 48 mg/kg bw No differences found in body weight, histology, haematology, urine analysis, blood biochemistry Sporadic, not dose-dependent differences reported in food efficiency, blood biochemistry, urinalysis and absolute and relative organ weights. Due to absence of findings in both sexes and/or the lack of a dose-response relationship, and the absence of histological alterations in the liver and kidneys, observed differences deemed not to be toxicologically relevant.
Schauss 2007, in English	Food consumption, animal and organ weight, haematology, blood biochemistry, histopathology	Chicken cartilage preparation	Rats	0, 30, 300, 1000 (10% HA) = 100 (highest)	NS	90 days	NOAEL= 100 mg/kg bw NaHA No dose-related or toxicologically significant changes in haematological parameters Small changes in the biochemical analyses considered unrelated to treatment due to the absence of a dose relationship
Canut 2012, in English	Animal and organ weight, food intake and behaviour, haematology, blood biochemistry, urinanalysis, histopathology	RCE	Rats	0, 5, 55, 600 (60-80% HA) =360-480 (highest)	800 kDa	90 days	NOAEL= 360 mg/kg bw No differences found (minor differences observed between treated and control animals in body-weight gain but there was no relation to dose)

Publication	Endpoints	Origin HA	Animal	Dose mg/kg bw/day	MW	Duration	Reported results
Chinese Center for Disease Control and Prevention 2007 (unpubl.)	Body weight gain, food consumption, organ weight, haematology, blood biochemistry, hisopathology	Ferment. NaHA	Rats	0, 330, 670 or 1000	670 kDa	90 days	NOAEL = 1000 mg/kg bw No differences found in weight gain, organ weight, biochemistry, some sporadic difference in biochemistry but not related to dose
Guo 2010, in Chinese	NS	Ferment.	Rats	1000	NS	90 days	NOAEL 1000 mg/kg bw No differences found
Tan 2008, in Chinese	NS	Ferment.	Rats	1333	NS	90 days	NOAEL 1333 mg/kg bw No differences found
Zuo 2008, in Chinese	Food consumption, animal and organ weight, haematology, blood biochemistry, histopathology	Ferment. NaHA	Rats	0, 667, 1000, 1333	NS	90 days	NOAEL= 1333 mg/kg bw No differences found in body weight gain, behaviour, No significant differences in haematological or histological and macroscopic parameters

BW=body weight, Ferment.=fermentation; LOAEL= lowest observed adverse effect Level; NaHA=sodium hyaluronate; NOAEL= no observed adverse effect Level; MW=molecular weight; NS=not specified; RCE=Rooster comb extract

4.5.2.4 Conclusion – animal toxicity studies

Sporadic differences in weight gain were observed in two studies, but for one study this could not be attributed to the treatment. Furthermore, this could not be repeated and was not seen for higher exposure doses in other studies. There were no dose related effects of HA exposure reported in the animal studies. In subchronic 90-day animal studies a NOAEL would be equal to the highest tested dose of 1333 mg/kg bw as reported in two separate studies.

4.5.2.5 Study characteristics – human RCTs

A total of 17 RCTs published from 2002 to 2021 were included in the identification and characterisation of potential adverse health effects of oral HA supplementation (Sato et al., 2002; Kalman et al., 2008; Hayashi et al., 2009 (Japanese); Sato et al., 2009; Nagaoka et al., 2010; Hunan Provincial Center for Disease Control and Prevention 2012 (cited in GRAS Assoc. 2020 as "unpublished report"); Tashiro et al., 2012; Martinez-Puig et al., 2013; Jensen et al., 2015; Kawada et al., 2015; Nelson et al., 2015; Sola et al., 2015; Takamizawa et al., 2016 (Japanese); Oe et al., 2017; Cicero et al., 2020; Hsu et al., 2021; and Michelotti et al., 2021). The two studies published in Japanese were translated into English.

Study design and indications for treatment: All studies reported to be double-blind with a parallel-group design, mostly with two study arms comparing HA supplementation to placebo, but three trials had three study arms to test HA of different molecular weights (Kawada et al., 2015, Oe et al., 2017), or different doses (Hayashi et al., 2009), against placebo. Most studies were designed to study effects of HA supplementation on different clinical symptoms or disease. The most common indications for HA supplementation were knee osteoarthritis or knee pain (nine studies), followed by skin conditions such as dry skin and skin ageing (five studies), and diverse chronic pain conditions (one study). The remaining two studies had safety as outcome. The HA supplement descriptions with dose and duration for the different studies is presented in Table 4.5.2.5-1 (grouped by indication for taking the supplement and manufacturing company).

Study dose and duration (Table 4.5.2.5-1): Overall, study durations ranged from 4 to 52 weeks, with a median of 8 weeks. The doses of HA ranged from around 25 mg to 225 mg HA per day. The highest dose of around 200 mg HA/day was assessed in at least five studies (Sato et al., 2009; Tashiro et al., 2012; Jensen et al., 2015; Cicero et al., 2020; Michelotti et al., 2021) and administered for durations ranging from 4 to 52 weeks. In some cases, the dose of HA is uncertain or approximate due to unspecified HA content of the product, or the HA dose is reported as a range (min-max) reflecting the reported range of HA in extracts from rooster- and chicken comb. In a sixth study (Hayashi et al., 2009), the HA dose was difficult to determine from the publication and was estimated to be in the range of 100-240 mg/day. In the study, "excess intake" was defined as 24 capsules, three times the regular intake (8 capsules), but the product name or HA content was not found. The manufacturing company was Everlife Co., Japan.

In other studies of supplements by Everlife Co. it was reported that HA was derived from chicken comb and that 6 capsules of Kojun premium® provided 25.2 mg HA (Takamizawa et al., 2016), whereas 6 capsules of Kojun® provided 630 mg/day of chicken comb extract (CCE), equivalent to approximately 60 mg HA (Nagaoka et al., 2010). Thus, in the study by Hayashi et al., 2009, one capsule may be assumed to contain from 4.2 to 10 mg HA, and the highest dose of 24 capsules would provide from 100 to 240 mg HA, given that the product from Everlife Co. had a similar HA concentration. Of note, some of the products (Oralvisc® by Bioibérica and Kojun® by Everlife Co.) are reported to be mixtures of HA with other compounds.

Manufacturing companies (Table 4.5.2.5-1): HA supplements form Asian manufacturers were dominating; Kewpie Co., Japan (six studies), Everlife Co., Japan (three studies), followed by Bioibérica, Spain (four studies), Roelmi HPC, Italy (two studies), and Viscos LLC., Fortville/IN, USA (one study) and one unknown manufacturer, but probably Chinese. The studies were characterized by a high degree of corporate funding and/or study authors with corporate affiliations (results not shown). Conflicts of interest (COI statements) apart from study funding by the manufacturer, were not reported for any of the studies. However, studies in which results on safety outcomes were shown rather than just stated by the authors (as indicated in next section 4.5.2.6, Table 4.5.2.6-1), were given more weight by VKM.

Study populations: The studies were predominantly (10 of 17) conducted in Asian study populations (Japan eight, Taiwan one, China one), followed by Europe (Italy two, and Spain two), and USA (three studies). Safety was assessed in both adult men and women, except three studies that only included women (two of skin conditions and one safety study). Study size has been reported by VKM as numbers randomized to intervention or placebo, as well as the number analysed (Section 4.5.2.6, Table 4.5.2.6-1Table 4.5.2.6-1). Numbers may differ due to participant drop-out or exclusions during analysis. Study size (randomized) ranged from around 20 to 100 with about half receiving the HA supplement in trials with two arms. The safety assessment was often based on the sample analysed (per-protocol analysis) if participants were lost.

Table 4.5.2.5-1 Randomized controlled trials (RCTs) addressing the safety of HA supplementation: product information, dose and treatment duration grouped by indication for treatment and supplement producer

Indication treatment	Producer	Product	Daily dose (product and HA)	Product details	Approximate HA dose (mg/day)	Duration	Publication
Knee osteoarthritis	Bioibérica, Spain	Hyal-Joint®	80 mg/day, equivalent to 48-56 mg HA/day, one capsule after breakfast	HA extracted from chicken combs containing 60-70% HA, MW not specified	48-56	8 wks	Kalman 2008
Knee osteoarthritis	Bioibérica, Spain	Oralvisc®	80 mg/day, equivalent to 56 mg HA/day	HA (70%) derived from rooster comb with other glycosaminoglycans, MW not specified	56	12 wks	Nelson 2015
Knee osteoarthritis	Everlife Co., Japan	Kojun®	630 mg/day equivalent to 60 mg HA/day.	HA derived from chicken combs with other compounds: propolis, xantho-oligo-sugar, vitamins (B1, B6, B12, E), iron and calcium	60	16 wks	Nagaoka 2010
Knee osteoarthritis	Everlife Co., Japan	Kojun premium®	25.2 mg HA/day, 6 tablets taken after breakfast	HA derived from chicken comb with other components	25.2	12 wks	Takamizawa 2016
Knee osteoarthritis	Kewpie Co., Japan	Hyabest (J)	200 mg/day (98.5% HA), 3 capsules taken after breakfast	HA by microbial Ferment., MW not specified	200	8 wks	Sato 2009
Knee osteoarthritis	Kewpie Co., Japan	Hyabest (J)	200 mg HA/day, 4 capsules taken after breakfast	MW 900 k, purity 97%	200	12 months (52 wks)	Tashiro 2012

Indication treatment	Producer	Product	Daily dose (product and HA)	Product details	Approximate HA dose (mg/day)	Duration	Publication
Knee osteoarthritis	Roelmi HPC, Italy	ExceptionHYAL® Jump, cotaining FS-HA®	200 mg/day supplement, HA content not specified	Full-spectrum sodium hyaluronate, broad range of MW (not specified)	200	60 days (8.6 wks)	Cicero 2020
Knee pain, mild	Bioibérica, Spain	Mobilee TM	80 mg/day supplemented in yoghurt, equivalent to 52 mg HA/day	HA extracted from rooster comb containing 65% HA, MW not specified	52	12 wks	Martinez-Puig 2013
Knee pain, mild	Bioibérica, Spain	Mobilee®	80 mg/day in 125 ml low fat yoghurt, equivalent to 52 mg HA/day	HA (65%) from rooster comb extract	52	12 wks	Sola 2015
Pain conditions, chronic	Viscos, LLC. (Fortville, IN, USA)	Liquid formula, no product name	225 mg HA/day (three tablespoons, 45 mL) during first 2 wks and 150 mg/day (two tablespoons, 30 mL) last 2 wks	Microbial fermentate (5 mg HA/ml), high MW hyaluronan (average between 2.5 and 2.8 million Daltons), flavored with sucralose and mild raspberry	225	4 wks	Jensen 2015
Safety study	Everlife Co., Japan	HA supplement, no brand name	24 capsules (high dose), 8 capsules (standard dose), placebo. HA content not specified.	Low molecular HA, MW not specified. 24 capsules equivalent to 100-240 mg/HA and 8 capsules 33-80 mg/HA per day based on other supplements from Everlife Co. (Kojun premium or Kojun, respectively)	100-240	8 wks	Hayashi 2009

Indication treatment	Producer	Product	Daily dose (product and HA)	Product details	Approximate HA dose (mg/day)	Duration	Publication
Safety study	Unknown, probably Chinese	Unknown product	100 mg NaHA/day	NaHA (MW=330 kDa) in mixture with unspecified oligopeptide & procyanidine	100	45 days (6.4 wks)	Hunan Provincial Center for Disease Control and Prevention 2012
Skin condition, dry skin, and wrinkles	Kewpie Co., Japan	Hyabest®(S) LF-P	120 mg/day taken as one capsule	HA, 95% purity	120	12 wks	Hsu 2021
Skin condition, dry skin	Kewpie Co., Japan	Hyaluronsan HA-F or Hyabest® (S) LF-P	120 mg HA/day, two capsules after breakfast	MW 800 k and 300 k, respectively	120	6 wks	Kawada 2015
Skin condition, wrinkles	Kewpie Co., Japan	Hyabest® (A) or Hyabest® (S) LF-P	120 mg HA/day as two capsules	HA of 95% purity, mean MWs were ~2 k and 300 k, respectively	120	6 wks	Oe 2017
Skin condition, dry skin	Kewpie Co., Japan	Hyaluronsan HA-F	120 mg HA/day given as one capsule twice per day after meals	Not specified	120	4 wks	Sato 2002
Skin condition, ageing skin (mild to moderate)	Roelmi HPC, Italy	ExceptionHYAL® Star	200 mg HA/day in the morning with a glass of water	Full-spectrum (broad range of MW, but not specified)	200	4 wks	Michelotti 2021

4.5.2.6 Results – human RCTs

None of the 17 RCTs reported adverse health effects that could be attributed to HA supplement intake (Table 4.5.2.6-1). In most studies the participants underwent physical examinations in a clinical study setting, but results on safety were often stated without showing supporting data or statistics. In these cases, VKM had to rely on author conclusions. In studies of skin conditions, the safety assessment was in some cases limited to skin reactions. Four studies (three Japanese and one U.S. study) included detailed assessments of safety (Sato et al., 2002; Hayashi et al., 2009; Jensen et al., 2015; Takamizawa et al. 2016). The assessment was based on physical examinations (body weight and/or body mass index, blood pressure, heart rate), registration of adverse reactions/events, blood tests and urine tests. Measurement during follow-up were compared to baseline values. Among these studies, the daily HA doses tested were 25.5 mg (Takamizawa et al., 2016), 120 mg (Sato et al., 2002), and 225 mg (Jensen et al., 2015). The dose used was uncertain in Hayashi et al. (2009) but estimated to be in the range of 100-240 mg/day as described previously. In all 17 studies, the estimated number of participants receiving HA supplements (sum of participants in the intervention groups) is around 490 based on the numbers analysed (Table 4.5.2.6-1).

Table 4.5.2.6-1 Results from RCTs among humans addressing safety of HA supplementation (sorted by dose and duration from low to high).

Daily dose, duration, publication	Study popul., M=Men W=Women	Study sample: randomised (analysed)	Safety assessment	Result	Reporting
Dose 25,2 (mg), dur 12 wks: Takamizawa 2016	Japan, M & W age ≥ 40 yrs, paid volunteers recruited publicly	80 (57.5% female): intervention 40 (38) and placebo 40 (39)	Biomarkers (blood and urine) at 4, 8, 12 wks and post intervention 16 wks, and participant report of adverse events	Fluctuations in biomarkers, but within physiological reference range. 6 adverse events in 5 of 80 participants (cold/infection), unrelated to intervention	Results shown, clinical examination, blood tests, adverse events
Dose 48-56 (mg), dur 8 wks: Kalman 2008	USA, M & W age ≥ 40 years with symptomatic knee OA, any race	20 (55% female): 11 intervention, 9 placebo	Physical examination (body weight, blood pressure, heart rate), blood test (complete blood cell count, and biochemical profile, not specified) and reported adverse events	Three adverse event, one acute non-target knee pain (intervention) unrelated to the study product, and two in placebo group (one diarrhoea and one hypoesthesia of the tongue). No significant changes in vital signs, body weight, and results of laboratory tests.	Results stated
Dose 52 (mg), dur 12 wks: Martinez-Puig 2013	Spain, M & W 50-75 (mean 59.6) yrs with symptomatic joint discomfort	40 (% female, unknown): 20 (19) intervention and 20 (18) placebo	Physical examination (body weight, pulse rate and blood pressure)	No significant changes in body weight, pulse rate and blood pressure after eating unsupplemented or supplemented yoghurt.	Results stated
Dose 52 (mg), dur 12 wks: Sola 2015	Spain, M & W, mean age 42.5 yrs, outpatients	84 (80, 62.5% female): 40 intervention, 40 controls	Adverse events coded according to the Medical Dictionary for Regulatory Activities (MedDra dictionary; version 9), no further specification	Nine mild adverse events related to gastrointestinal discomfort such as flatulence and stomach ache. No statistically sig difference between groups.	Results stated

Daily dose, duration, publication	Study popul., M=Men W=Women	Study sample: randomised (analysed)	Safety assessment	Result	Reporting
Dose 56 (mg), dur 12 wks: Nelson 2015	Japan, M & W 45-75 (mean 61) yrs, obese knee OA patients (mean BMI 34.6 kg/m2)	51 (40, 50% female): 25 (21) intervention, 26 (25) placebo	Not stated	No mention of nausea or other gastrointestinal complaints from preparation or placebo	Results stated
Dose 60 (mg), dur 16 wks: Nagaoka 2010	Japan, M & W age 40-85 yrs, diagnosed with OA	43 (81% female): 21 intervention and 22 controls (11 doing exercise therapy in each group)	Reported adverse events and changes in body weight/BMI, blood pressure, pulse rate and laboratory tests, including haematology, biochemical profile and urinalysis	Total number of adverse events 45 in intervention group and 32 in the placebo group (no sig difference): cold symptoms (12 vs. 3); pain (6 vs. 6); myalgia (6 vs. 5); gastric discomfort (6 vs. 3); diarrhoea (3 vs. 4); and cramp (2 vs. 2). None considered severe or related to study diet. No sig change in body weight and BMI, blood pressure (systolic/diastolic), pulse rate, and laboratory tests (haematology and blood chemistry) from baseline in either of the groups	Results stated

Daily dose, duration, publication	Study popul., M=Men W=Women	Study sample: randomised (analysed)	Safety assessment	Result	Reporting
Dose 100 (mg), dur 45 days (6.4 wks): Hunan Provincial Center for Disease Control and Prevention 2012	China, W aged 30-50 yrs	104 (100% female): 52 intervention/52 placebo	Physical examination (weight, blood pressure, heart rate), biomarkers (blood, urine), stool examination, electrocardiogram, abdominal ultrasound & chest x-ray	No significant differences in weight, blood pressure or heart rate between groups. After 45 days, all urine analysis (pH, white blood cells, urine sugar), stool exam, hematologic (RBC, WBC, haemoglobin) & biochemical assay results (glucose, triglyceride, cholesterol, BUN, creatinine, uric acid, alanine aminotransferase, aspartate aminotransferase, total protein, albumin) were within the normal range. No abnormalities observed in electrocardiogram, abdominal ultrasound or chest x-ray	Results reported in GRAS Assoc. (2020)
Dose 100-240 (mg), dur 8 wks: Hayashi 2009	Japan, M & W age > 20 (mean 38) yrs, healthy paid volunteers	44 (50% female): 16 (14) high dose, 16 (15) standard dose, 12 placebo	Physical examination (body weight, body fat, blood pressure, pulse), biomarkers (blood, urine) and medical interview	Fluctuations in blood test values in HA group were minimal, within the reference range, and a similar trend was observed in the placebo group. Most adverse events were common cold symptoms due to cold season not found to be causally related to HA	Results shown, clinical examination, blood tests, urine tests, adverse events

Daily dose, duration, publication	Study popul., M=Men W=Women	Study sample: randomised (analysed)	Safety assessment	Result	Reporting
Dose 120 (mg), dur 4 wks: Sato 2002	Japan, M &W, mean age 31.5 yrs, paid, healthy volunteers recruited publicly	35 (63% female) analysed: 17 intervention and 18 placebo	Blood test at baseline and 4 wks: blood cell components (white and red blood cell count, haemoglobin, haematocrit, MCV, MCH, MCHC, platelets), protein, albumin, A/G ratio, GOT, GPT, gamma-GPT, LDH, ALP, total bilirubin, creatinine, uric acid, BUN, Na, K, Cl, Ca, Mg, HbA1c, total chol, HDL cholesterol, triglycerides, globulin	All changes within normal range in both groups (increase in MCH, MCHC, K, Ca, and decrease in MCV in HA group, decrease in haemoglobin in placebo group, decrease in haematocrit, globulin and increase in HbA1c, Na and Cl in both groups). Only sig difference between groups was in Ca.	Results shown, blood test
Dose 120 (mg), dur 6 wks: Kawada 2015	Japan, W age 35-60 (mean 43) yrs, healthy volunteers	66 (61,100% female): 19 intervention 1 (HA 800 k), 20 intervention 2 (300 k), 22 placebo	Clinical examination of adverse skin reactions (face and whole body) by hospital dermatologist at 2, 4, 6 wks and 2 wks post treatment	Slight dry skin, rough skin, and poor skin lustre observed at 3 wks in one subject in the placebo group. No adverse effects on skin reported by dermatologist	Results stated
Dose 120 (mg), dur 6 wks: Oe 2017	Japan, M & W age 22-59 yrs, healthy volunteers	60 (50, 60% female): 20 (16) intervention 1 (2 k HA), 20 (16) intervention 2 (300 k HA), 20 (18) placebo	Questionnaire survey on adverse effects, such as allergic reactions, diarrhoea, and abdominal pain	No adverse events	Results stated
Dose 120 (mg), dur 12 wks: Hsu 2021	Taiwan, M & W age 30-65 (mean 44) yrs, volunteers	40 (72.5% female): 20 intervention, 20 placebo	Not described	No adverse events attributable to the consumption of HA	Results stated

Daily dose, duration, publication	Study popul., M=Men W=Women	Study sample: randomised (analysed)	Safety assessment	Result	Reporting
Dose 200 (mg), dur 4 wks: Michelotti 2021	Italy, W age 35-70 (mean 54.5) yrs, healthy	60 (100% female): 30 intervention and 30 placebo	Clinical assessment by dermatologist, self-reported tolerance	No side effects or adverse events reported throughout the study	Results stated
Dose 200 (mg), dur 8 wks: Sato 2009	USA, M & W ≥ 40 yrs	40 (37, 78% female): 20 intervention and 17 placebo	Blood tests at baseline and 8 wks: blood cell's components (white cell count, red cell count, haemoglobin, haematocrit), urea nitrogen, creatinine, AST(GOT), ALT(GPT), alkaline phosphatase, blood sugar level, sodium, and potassium	No significant changes between HA and placebo group, nor any adverse event attributable to HA group throughout period. Statistically sig. increase of alkaline phosphatase and decrease of ALT(GPT) in placebo group and decrease of white blood cell count in both groups at 8 weeks not found clinically important.	Results stated
Dose 200 (mg), dur 60 days (8.6 wks): Cicero 2020	Italy, M & W age 30-85 yrs, treated for knee OA	60 (50, 54% female): 30 (25) intervention, 30 (25) placebo	Physical examination at baseline and wks 4 and 8 and assessment of tolerability at wks 4 and 8, no further specification	All subjects completed trials without any clinically detectable adverse events registered in either group of treatment	Results stated
Dose 200 (mg), dur 12 months (52 wks): Tashiro 2012	Japan, M & W age ≥ 50 yrs, seeking treatment at orthopaedic clinics	60 (77% female): 30 intervention (18) and 30 placebo (20) groups	Recording of side effects, methods not specified	Possible side effects in HA groups were gastric discomfort in 2 subjects, reflux esophagitis in 1, and appetite loss in another. In the placebo group, skin irritation (itching or skin redness) was found in 2 subjects. Differences not statistically sig. Problems not demonstrated to be caused by the ingestion of HA or placebo.	Results stated

Daily dose, duration, publication	Study popul., M=Men W=Women	Study sample: randomised (analysed)	Safety assessment	Result	Reporting
Dose 225 (mg), dur 4 wks: Jensen 2015	USA, M & W age 22-71 yrs	78 (67% female): 39 (37) intervention, 39 (35) placebo	Blood tests (screening, 2 wks and 4 wks) for complete blood count and differential count, comprehensive metabolic panel with hepatic function, heart function (3-lead ECG) at 2 wks and 4 wks	No major change in blood cell counts, glomerular filtration rate or heart function. Statistically sig. but small decrease within HA group for blood levels of sodium, CO_2, blood urea nitrogen, creatinine, and albumin	Results shown, metabolic panel with liver function

ALT(GPT)=alanine aminotransferase; A/G ratio=albumin/globulin ratio; ALP=alkaline phosphatase; AST(GOT)=aspartate aminotransferase; BUN=blood urea nitrogen; Ca=calcium; Cl=chloride; HbA1c=glycated haemoglobin; LDH=lactate dehydrogenase; Mg=magnesium; MCH=mean corpuscular haemoglobin; MCHC=mean corpuscular haemoglobin concentration; MCV=mean corpuscular volume; K=potassium; RBC=red blood cells; Na=sodium; WBC=white blood cells

4.5.2.7 Conclusion based on the results from RCTs

No adverse effects were reported in the reviewed RCTs among humans with exposure up to 200 mg/day for 12 months. However, the duration and study size were too limited for any identification of potential long-term or rare events at the population level. In the sub-set of studies with measured biomarkers in blood and urine, changes were within the reported reference ranges in both the intervention and placebo groups and not considered clinically relevant.

4.6 Summary of the hazard identification and characterisation

VKM's hazard identification and characterization is based on several lines of evidence combined:

- 1) Endogenous production
- 2) Absorption, distribution, metabolism, and excretion (ADME)
- 3) Genotoxicity and reproductive toxicity
- 4) Animal toxicity studies
- 5) Human randomized controlled trials (RCTs)
- 6) Previous risk assessments from competent bodies

Hyaluronic acid is endogenously produced with a daily turnover of 5000 mg/day. It is metabolised to innocuous fragments and only smaller fragments (< 2kDa) appears to be absorbed directly, while larger variants must be metabolised before uptake. Information on absorption, distribution and excretion is conflicting possibly due to differences in design and test methodology used. It ranges from no significant absorption and only traces detected in blood following oral administration regardless of molecular weight and up to a bioavailability of 16% of HA in plasma at 90% absorption, and a few percentages detected in bone, joints, muscle, and skin. HA is metabolised by hyaluronidases by microbiota and in the tissue and further by glucosidases into monosaccharides and CO_2 in the liver. Hyaluronic acid is not considered genotoxic.

Based on animal studies (information extracted from GRAS Assoc. 2020, Oe et al., 2016, and Kawada et al., 2014) the highest tested single oral dose in mice of 15 000 mg NaHA/kg bw did not result in deaths or obvious toxic symptoms during the study period of 14 days. For HA from RCE the highest tested dose in a 90-day repeated dose study was 600 mg RCE (equal to 360-480 mg NaHA/kg bw/day), and for fermented NaHA it was 1333 mg/kg bw/day and no toxic effects were observed in these studies. The highest doses tested in reproductive and developmental toxicity studies in rats did not show adverse effects and revealed a NOAEL of 670 mg/kg bw/day and no teratogenic effect detected after 1333 mg/kg bw/day of NaHA.

A small number of reports on allergenicity have all been negative. No antigenicity was detected after intraperitoneal administration of 100 ug/kg (GRAS Assoc. 2020). In confidential technical information submitted to the NFSA for a product based on RCE, tests by the applicant showed no cross reactivity with egg proteins in serum from egg allergic participants.

The overall NOAEL of 1333 mg/kg bw of NaHa obtained in subchronic studies in rats (considering fermented NaHA and NaHA from RCE equivalent) was used as a reference point to characterize the hazard.

The longest human oral exposure study lasted for 12 months with 200 mg/day of NaHA with no adverse events registered related to HA. The reporting on how possible adverse events were investigated and recorded was limited in many studies, but a sub-set of four studies reported an extensive list of biomarkers. In this sub-set of studies with clinical measurements, the highest daily dose that could be established with certainty was 225 mg/day for 2 weeks, followed by 150 mg/day for 2 weeks with no adverse effects registered. All reported data on biomarkers were within the reference ranges. Oral intake of around 200 mg/day has not been associated with adverse effects based on six human RCT studies, with around 150 or 180 participants in total receiving this dose. Two of 17 studies reported sporadic side effects: gastric discomfort, reflux esophagitis, and appetite loss after 12 months of 200 mg/day oral HA intake or 48 mg HA from RCE for 12 weeks. Due to few events and similar effects in the placebo group the side effects were not possible to attribute to HA intake according to the study authors.

The reported RCTs are in general small for assessing potentially rare adverse effects at the population level, and the duration (only one study lasting up to 12 months) may not capture long-term adverse effects. Hence, there is limited evidence for absence of any adverse health effects in humans.

5 Risk characterisation

Based on the available evidence VKM concludes that a daily intake of food supplements with 150 mg, 120 mg, 64 mg, and 48 mg HA is well within daily levels of endogenous production and excretion.

In the risk characterization VKM used the reference point of 1333 mg/ kg bw obtained in the hazard characterization for comparison with the human doses 2.9, 2.3, 1.3 and 0.9 mg/kg bw for the 5th percentile for human intake of HA supplements, see Table 3.1-1) and to calculate a margin of exposure (MOE).

The human oral exposure of 2.9, 2.3, 1.2 and 0.9 mg/kg bw of HA doses (corresponding to 150, 120, 64 and 48 mg/day) were compared to the refence point of 1333 mg/kg bw. A margin of exposure (MOE) of 460 (1333/2.9) to the highest dose was then obtained.

In support of the evidence from animal studies of no adverse effects of the given doses, no adverse effects have been reported in human RCTs.

6 Uncertainties

- Data on absorption and bioavailability of HA is conflicting and supplement doses have been evaluated without any absorption adjustments.
- Absorption is highly dependent on molecular weights of HA, which is often not reported.
- It is uncertain to what extent oral exposure in the range of 150 mg can affect a daily endogenous production of 5000 mg.
- Research on the health effects of HA supplements has mainly been conducted in RCTs to assess potential beneficial effects on conditions such as knee osteoarthritis/joint pain or dry skin and wrinkles. The study sizes and duration of HA supplement intake may be insufficient to capture rare adverse effects and adverse long-term effects.
- Potential interactions between HA and other substances have not been studied

7 Conclusions (with answers to the terms of reference)

VKM has assessed whether hyaluronic acid (CAS number 9067-32-7) in food supplements intended for adults aged 18 years and older may pose a health risk in the Norwegian population, in a daily dose of 150 mg, 120 mg, 64 mg, and 48 mg hyaluronic acid.

• Identification of adverse health effects (hazard assessment):

- ✓ HA is a natural compound in the human body. The estimated daily turnover of HA in humans is about 5000 mg. HA in the body is degraded by hyaluronidases to innocuous fragments that enters endogenous metabolism.
- ✓ Information on absorption, distribution and excretion is limited and conflicting and based on animal studies. In animals, smaller fragments (< 2 kDa) appear to be absorbed directly, while larger variants must be degraded by intestinal microflora before uptake. Radiolabelled HA appears to be widely distributed in experimental animals and 75%-90% is reported to be excreted within 72 hours.
- ✓ No harmful health effects have been identified in animal studies.
- ✓ Human RCTs do not report any adverse health effects attributed to HA intake, but the studies often provide sparse information on how potential adverse effects were investigated. VKM had to rely on author conclusions in many studies, due to limited reporting of results.
- ✓ The over-all NOAEL in animal studies was set to the highest dose investigated in long term studies (1333 mg/kg bw) and used as reference point to characterize the hazard.

• Evaluation of the health risk of the exposure (risk characterisation):

- ✓ Exposures to HA from the food supplement for various age groups are shown in Table 3.1-1. It is noted that information on oral exposure to HA from other sources is lacking as HA content of common food sources except from rooster comb is unavailable.
- ✓ The human oral exposures of 2.9, 2.3, 1.2 and 0.9 mg/kg bw corresponding to HA doses of 150, 120, 64 and 48 mg/day for the P_5 weight percentile, respectively, were compared to the refence point of 1333 mg/kg bw. A margin of exposure (MOE) range from 460 to 1480 was then obtained.
- ✓ The MOE of 460 based on subchronic studies in rats is considered sufficiently large to indicate that no known health risks are associated with the suggested exposure to hyaluronic acid.

- ✓ Evidence from 17 human RCTs support the conclusion from animal studies that the exposure to the given doses of HA food supplements is unlikely to cause adverse effects. None of the RCTs exceeded 12 months duration.

8 Data gaps

- HA is a common natural component of animal foods, still no data has been found on background exposure to natural HA from the diet.
- Human RCTs of effects of HA exceeding 12 months were not found.
- Human studies on ADME of HA are lacking.

Appendix I

Literature search strategy review studies (human/animal)

HYALURONSYRE

Kontaktperson:	Bente Mangschou
Søk:	Ragnhild Agathe Tornes
Fagfelle:	Astrid Merete Nøstberg
Dublettsjekk i EndNote:	Før dublettkontroll: 3848 Etter dublettkontroll: 1976

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations, Daily and Versions <1946 to December 30, 2022>
Dato: 03.01.23
Antall treff: 847

1	Hyaluronic Acid/	25085
2	(hyaluronic acid or hyaluronan or hyaluronate).tw,kf.	36267
3	1 or 2	40083
4	limit 3 to "reviews (maximizes specificity)"	695
5	Meta-Analysis/ or Network Meta-Analysis/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kf,bt.	477484
6	4 or (3 and 5)	847

Database: Embase <1974 to 2022 December 30>
Dato: 03.01.23
Antall treff: 1330

1	hyaluronic acid/	50141
2	(hyaluronic acid or hyaluronan or hyaluronate).tw,kf.	45096
3	1 or 2	58516
4	limit 3 to "reviews (maximizes specificity)"	1053
5	exp Meta-Analysis/ or "systematic review"/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kf.	693959
6	4 or (3 and 5)	1704
7	limit 6 to embase	1330

Database: Cochrane Database of Systematic Reviews
Issue 1 of 12, January 2023
Dato: 03.01.23
Antall treff: 28

#1	[mh ^"Hyaluronic Acid"]	1903
#2	("hyaluronic acid" or hyaluronan or hyaluronate):ti,ab	4195
#3	#1 or #2	4551
#4	#3 in Cochrane Reviews	28

Database: Web of Science Core Collection
A&HCI , ESCI , SCI-EXPANDED , SSCI
Dato: 03.01.23
Antall treff: 997

3	#1 AND #2 | Exact search	997
2	TS=(("systematic*" NEAR/1 "review*") or ("review" and (("structured" or "database*" or "systematic*") NEAR/1 "search*")) or "integrative review*" or ("evidence" NEAR/1 "review*")) OR TI=("metaanal*" or "meta anal*") OR AB=("metaanal*" or "meta anal*") | Exact search	531,112
1	TS=("hyaluronic acid" or hyaluronan or hyaluronate) | Exact search	47,663

Database: Epistemonikos
Dato: 03.01.23
Antall treff: 646

Title/Abstract: ("hyaluronic acid" or hyaluronan or hyaluronate)
Publication type: Systematic Review

Literature search strategy human RCTs

HYALURONIC ACID, ROOSTER COMB EXTRACT AND CHICKEN COMB EXTRACT

Kontaktperson:	Bente Mangschou
Søk:	Ragnhild Agathe Tornes
Fagfelle:	Astrid Nøstberg
Dublettsjekk i EndNote:	Før dublettkontroll: 1651 Etter dublettkontroll: 1122

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations, Daily and Versions <1946 to January 12, 2023>
Dato: 13.01.23
Antall treff: 466

1	Hyaluronic Acid/	25203
2	(hyaluronic acid or hyaluronan or hyaluronate or ((rooster or chicken) adj comb extract*)).tw,kf.	36351
3	1 or 2	40159
4	Dietary Supplements/ or Diet/ or Food/ or Eating/	323544
5	(oral or diet* or supplement? or intake or ingestion? or eat*).tw,kf.	1673695
6	4 or 5	1766643
7	3 and 6	1614
8	limit 7 to "therapy (maximizes specificity)"	133
9	("randomized controlled trial" or "controlled clinical trial").pt. or (randomized or randomised or randomly or rct or placebo or trial or groups).tw,kf,bt.	3658858
10	8 or (7 and 9)	466

Database: Embase <1974 to 2023 January 12>
Dato: 13.01.23
Antall treff: 704

1	hyaluronic acid/	50260
2	(hyaluronic acid or hyaluronan or hyaluronate or ((rooster or chicken) adj comb extract*)).tw,kf.	45198
3	1 or 2	58654
4	dietary supplement/ or exp diet/ or exp food/ or food intake/ or eating/	1564673
5	(oral or diet* or supplement? or intake or ingestion? or eat*).tw,kf.	2182116
6	4 or 5	3121899
7	3 and 6	3958
8	limit 7 to "therapy (maximizes specificity)"	342
9	limit 7 to (randomized controlled trial or controlled clinical trial)	281
10	(randomized or randomised or randomly or rct or placebo or trial or groups).tw,kf.	4920594
11	8 or 9 or (7 and 10)	1088
12	limit 11 to embase	704

Database: **Cochrane Central Register of Controlled Trials**
Issue 12 of 12, December 2022
Dato: 13.01.23
Antall treff: 481

#1	[mh ^"Hyaluronic Acid"]	1903
#2	("hyaluronic acid" or hyaluronan or hyaluronate or (("rooster comb" or "chicken comb") NEXT extract*)):ti,ab	4195
#3	#1 or #2	4551
#4	[mh ^"Dietary Supplements"]	12258
#5	[mh ^Diet]	7596
#6	[mh ^Food]	1352
#7	[mh ^Eating]	3033
#8	(oral or diet* or supplement? or intake or ingestion? or eat*):ti,ab	286795
#9	(Hsu et al., -#8)	292703
#10	#3 and #9	490
#11	#10 in Trials	481

List of excluded studies

Eight human studies (six from the main search and two from the citation search, Figure 4.5.1.8-1) were excluded after full text screening according to the eligibility criteria. The exclusion reasons are specified below for each study. Two publications in Japanese could not be retrieved and assessed for eligibility.

Reference	Reason for exclusion
Bellar 2019	Exclude/not RCT
Fiorino 2019	Exclude/HA as add-on therapy
Kim 2019	Exclude/no safety outcome
La Galia 2014	Exclude/no safety outcome
Moriña 2013	Exclude/conference abstract, covered by Moriña 2018
Moriña 2018	Exclude/no safety outcome
Sanchez 2014	Exclude/no safety outcome
Yoshimura 2012	Exclude/no safety outcome
	Not retrievable, not assessed for eligibility
Hatayama 2008	Not retrievable by library
Iwaso 2009	Not retrievable in time

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