National Institute for Public Health
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Ministry of Health, Welfare and Sport

Risk assessment of food supplements containing **N-acetylcysteine (NAC)**

RIVM letter report 2024-0086
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Colophon

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Synopsis

Risk assessment of food supplements containing N-acetylcysteine (NAC)

Food supplements containing the substances N-acetylcysteine, L-cysteine and/or L-cystine are sold in the Netherlands. These supplements are intended to detoxify the body. The Dutch Ministry of Health, Welfare and Sport has asked the National Institute for Public Health and the Environment (RIVM) to assess the safety of these food supplements.

Adults can take up to a maximum of 1,200 milligrams of N-acetylcysteine or 900 milligrams of L-cysteine or L-cystine per day without harmful effects. For children from the age of 2 to 18, the maximum quantity is lower. People who take more than the maximum quantity may develop gastrointestinal symptoms.

There are supplements on the market that recommend a higher dose than this maximum. That is why RIVM advises users to be careful not to take too much. RIVM recommends against giving these supplements to children under the age of 2.

N-acetylcysteine is also registered as an expectorant drug. Taking the food supplements together with this drug is not recommended, as people may take too much.

The Ministry asked RIVM to assess the safety of N-acetylcysteine. The human body converts N-acetylcysteine into the substances L-cysteine or L-cystine. As the supplements may also contain these substances, RIVM assessed all three of these substances.

Keywords: dietary supplements, cystine, safety, risk assessment

Publiekssamenvatting

Risicobeoordeling van voedingssupplementen met N-acetylcysteïne (NAC)

In Nederland worden voedingssupplementen verkocht waar de stof N-acetylcysteïne, L-cysteïne en/of L-cystine in zit. De supplementen zijn bedoeld om het lichaam te helpen ontgiften. Het ministerie van VWS heeft het RIVM gevraagd om de veiligheid van deze voedingssupplementen te beoordelen.

Volwassenen kunnen per dag maximaal 1200 milligram N-acetylcysteïne of 900 milligram L-cysteïne of L-cystine binnenkrijgen zonder schadelijke effecten. Voor kinderen van 2 tot 18 jaar is de maximale hoeveelheid lager. Als gebruikers meer dan de maximale hoeveelheid binnenkrijgen, kunnen ze er bijvoorbeeld maag-darmklachten van krijgen.

Er zijn supplementen op de markt die een hogere dosering aanraden dan dit maximum. Daarom raadt het RIVM gebruikers aan om erop te letten dat ze niet te veel binnenkrijgen. Het RIVM raadt aan deze supplementen niet te gebruiken voor kinderen onder de twee jaar.

N-acetylcysteïne is ook geregistreerd als slijmoplossend medicijn. Het wordt afgeraden om de voedingssupplementen tegelijk met dit medicijn te gebruiken, omdat mensen er dan te veel van kunnen binnenkrijgen.

VWS heeft het RIVM gevraagd om de veiligheid van N-acetylcysteïne te beoordelen. NAC wordt in het lichaam omgezet tot de stoffen L-cysteïne of L-cystine. Omdat deze twee stoffen ook in de supplementen kunnen zitten, heeft het RIVM alle drie de stoffen beoordeeld.

Kernwoorden: voedingssupplementen, cysteïne, veiligheid, beoordeling

Contents

Summary

Introduction

In December 2020, the Minister for Medical Care and Sport of the Ministry of Health, Welfare and Sport (VWS) announced the actions that would be taken to better regulate food supplements and herbal preparations in the Netherlands, thereby facilitating enforcement. One of those actions is to expand the list included in the Herbal Preparations Decree of the Dutch Commodities Act with substances/botanicals that are either forbidden or restricted (i.e. subject to a maximum limit) in food supplements or herbal preparations. In order to determine whether a substance or botanical needs to be included in this list, a risk assessment is warranted. The selection of substances and botanicals chosen for risk assessment was based on the prerequisites that the substances/botanicals were sold on the Dutch market and (widely) used in the Netherlands and there were indications for possible health risks, e.g. from Rapid Alert System for Food and Feed (RASFF) reports or from enforcement institutes. The current assessment is about food supplements containing N-acetyl-L-cysteine (NAC).

NAC is the N-acetyl derivative and precursor of the endogenous amino acid L-cysteine. In the body, NAC is metabolized to L-cysteine, and L-cysteine can be interconverted to/from its dimer L-cystine. Therefore, information on these two compounds is included in this assessment as well.

Currently, in the Netherlands, there are no specific restrictions for the use of NAC, L-cystine or L-cysteine in food supplements and/or herbal preparations included in the Herbal Preparations Decree of the Dutch Commodities Act[1]. NAC is registered in the Netherlands for medicinal use as mucolytic agent, paracetamol antidote or for treatment of keratoconjunctivitis sicca[2,3].

In Europe, L-cysteine is approved as a food additive (E920) in flour and biscuits for infants and young children, and may be present as a food additive in food enzymes. Both L-cysteine and L-cystine are authorized as flavouring agents.

Previous existing assessments

Several assessments of NAC, L-cysteine and L-cystine have been conducted in the past.

The European Food Safety Authority (EFSA, 2003) evaluated the use of NAC in foods for particular nutritional uses and in foods for special medical purposes (FSMPs). EFSA concluded that the proposed use of NAC for replacement of L-cysteine in foods for particular nutritional uses is not acceptable because it would exceed the therapeutic dose of 400 to 600 mg per day and, amongst others, gastrointestinal complaints could

[1] https://wetten.overheid.nl/BWBR0001969/2018-11-17, Accessed August, 2022.
[2] Dry eyes due to decreased tear production
[3] Home | Geneesmiddeleninformatiebank | College ter Beoordeling van Geneesmiddelen. Accessed July 2023

not be excluded in sensitive individuals. For FSMPs, EFSA concluded that the inclusion of NAC in the list of substances permitted is acceptable, given that FSMPs should be used under the supervision of a physician.

The Scientific Committee on Food considered the use of L-cysteine as flour treatment agent (food additive) toxicologically acceptable (SCF; 1991) and EFSA concluded it to be of no safety concern for infants and young children (EFSA, 2006).

The use of L-cystine and L-cysteine as flavouring agents was not of safety concern at their estimated levels of dietary exposure as concluded by the Joint FAO/WHO Expert Committee on Food Additives (JECFA; 2004) and/or EFSA (2007).

The Institute of medicine of the National Academy of Sciences of the United States (2005) concluded that the available data on the adverse effects of L-cysteine and L-cystine resulting from the use of supplements are not adequate for a dose-response assessment and the derivation of a tolerable upper intake level.

The French Agency for Food, Environmental and Occupational Health & Safety (ANSES; 2011) published an assessment of the risks associated with substances with nutritional or physiological effects and listed reported adverse effects of cysteine, including hypercholesterolemia, fatty liver and neurotoxicity but could not conduct a proper risk assessment for cysteine due to the complexity of amino acid metabolism, the scarcity of toxicological data, and the insufficiently characterized risks.

The Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition concluded that a maximum daily amount of 300 mg is acceptable from a safety point of view (AESAN, 2012; AECOSAN, 2015).

The Norwegian Scientific Committee for Food Safety (VKM; 2015) assessed the safety of the use of food supplements providing L-cysteine in a daily dose of 10 mg and of food supplements providing L-cystine in a daily dose of 250, 500, 750 and 1000 mg. Since there were only a few intervention studies with L-cysteine or L-cystine available, studies with NAC were considered as well for this assessment. VKM concluded that a dose of 1200 mg NAC per day, which yields doses of L-cysteine or L-cystine up to 900 mg per day (corresponding to 13 mg/kg bw per day for a 70 kg adult), does not lead to appreciable health risks in adults. There were no indications that children ≥10 years of age and adolescents are more vulnerable (on a mg/kg bw basis) than adults for these compounds. VKM concluded that the proposed doses of 10 mg per day L-cysteine and doses of 250, 500 and 750 mg per day L-cystine are considered to be unlikely to cause adverse health effects in adolescents (14 to 18 years) and adults (≥18 years), whereas the proposed dose of 1000 mg per day L-cystine may represent a risk of adverse health effects. For children (10 to 14 years) doses of 10 mg per day L-cysteine and doses of 250 and 500 mg per day L-cystine were considered to be unlikely to cause adverse health effects, whereas the doses of 750 and 1000 mg per day L-cystine may represent a risk of adverse health

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

The Swiss Federal Food Safety and Veterinary Office (2016) concluded that a specific food supplement containing a daily dose of 50 mg per day NAC intended for use by women of childbearing age was of no toxicological concern.

The current risk assessment uses the assessment of VKM as starting point supplemented with literature data that has been published since.

Food supplements on the Dutch market

Various food supplements containing NAC, L-cysteine, L-cystine or a combination of these compounds can be found on the Dutch market, mainly as capsules. The recommended dose generally varies from 1 to 3 capsules per day corresponding to recommended daily doses ranging from 200 to 2000 mg NAC, L-cysteine or L-cystine.

Exposure assessment

Based on their recommended use, the exposure to NAC resulting from the use of food supplements containing NAC will vary from 500 to 2000 mg per day, corresponding to 7.1 – 29 mg/kg bw per day for a 70-kg adult. This yields an exposure to L-cysteine of approximately 375 to 1500 mg[4], corresponding to 5.4 – 21 mg/kg bw per day for an adult of 70 kg.
The use of food supplements containing L-cysteine following the recommended use, results in exposures of 500 to 1500 mg L-cysteine per day, corresponding to 7.1 – 21 mg/kg bw per day for a 70-kg adult.
The use of food supplements containing L-cystine according to the recommended use results in exposures of 200 to 400 mg L-cystine per day, corresponding to 2.9 – 5.7 mg/kg bw per day for a 70-kg adult.

Some food supplements are also marketed for children. Exposure to NAC, L-cysteine and L-cystine by children due to the use of food supplements may occur in similar absolute amounts as for adults. Due to their lower bodyweights, the relative exposure per kg bodyweight will however be higher.

Additional exposure to NAC (or L-cysteine or L-cystine) may occur when people use NAC as mucolytic medicine together with supplements containing NAC, L-cysteine or L-cystine. The therapeutic dose for children >7 years and for adults is 600 mg per day NAC, corresponding to 450 mg per day L-cysteine. The therapeutic dose for children from 2 to 7 years is 400 mg per day NAC corresponding to 300 mg per day L-cysteine. The use is contraindicated for children < 2 years.

Further, additional exposure may result from the use of L-cysteine as food additive, the use of both L-cystine and L-cysteine as flavouring agents, and the fact that they are present in the normal diet as components of food proteins.

[4] Calculated by multiplying dose of NAC by ratio molecular weight L-cysteine – molecular weight NAC

Biological data

- Following oral exposure, NAC is rapidly absorbed in humans (Holdiness, 1991). The oral bioavailability of NAC is around 6-10% (Borgström, Kagedal & Paulsen, 1986). The terminal half-life of NAC is 6.25 hours following oral intake of 400 mg (Olssen et al., 1988). Besides extensive metabolism, elimination also takes place via renal clearance. Only a small part is excreted via faeces (Bonanomi & Gazzaniga, 1980). In patients with end-stage renal disease or severe liver injury the kinetics of NAC are altered due to diminished total clearance (Jones et al., 1997; Nolin et al., 2010).
- NAC, L-cysteine and L-cystine all have a low acute toxicity with oral median lethal dose values in mice and/or rats higher than 1890 mg/kg bw. (Bonanomi & Gazzaniga, 1980; Takasaki et al., 1973; Sprince et al., 1974; Kawai et al., 1978).
- Limited short-term and sub-chronic toxicity data are available. For NAC, no effects on behaviour, body weight gain, haematology, hepatic and renal function, prothrombin and bleeding time or pathological lesions upon histological examination and necropsy findings were observed in rats after a daily dose of up to 2000 mg/kg bw for 4 weeks or up to 1000 mg/kg bw for 12 and 28 weeks (Bonanomi & Gazzaniga, 1980). For L-cysteine, adverse effects [no further details provided] were observed at doses from 200 mg/kg bw per day onwards in mice and rats exposed for 30 days and in male rats exposed for 6 months to 100 mg/kg bw per day or higher (Takasaki et al., 1973). For cystine, a NOAEL of 600 mg/kg bw per day was identified [no further details] (Kawai et al., 1978).
- The available genotoxicity data do not provide indications that NAC, L-cysteine or L-cystine are genotoxic. However, due to limitations in the available data it is not possible to adequately assess the genotoxicity of these compounds.
- In a chronic toxicity study, no effects on behaviour, body weight gain, haematology, hepatic and renal function, prothrombin and bleeding time and no pathological lesions upon histological examination or necropsy findings were observed in dogs exposed to NAC at doses up to 300 mg/kg bw per day for 52 weeks (Bonanomi & Gazzaniga, 1980). No chronic toxicity study with L-cysteine or L-cystine or carcinogenicity studies were identified.
- A dose of 250 mg/kg bw NAC did not result in adverse effects on fertility in male rats, but at higher doses of 500 and 1000 mg/kg bw a slight reduction of fertility was observed (Bonanomi & Gazzaniga, 1980).
- No adverse effects were observed in developmental toxicity studies in rats or rabbits at doses of NAC up to 2000 mg/kg bw (Bonanomi & Gazzaniga, 1980). In a six generation study on reproductive toxicity a NOAEL of 3500 mg/kg bw L-cysteine was found, the only dose tested (Frape et al., 1971).
- With respect to effects on cholesterol, animal studies seem to suggest that high doses of NAC, approximately approximately a factor of 10 higher than human doses may interfere with the fatty acid metabolism and lead to hepatotoxicity while at low levels this negative effect is not observed (Niewiadomski et al.,

2016; Liou et al., 2021; Hang et al., 2021). Human studies suggest that high plasma levels of cysteine are correlated with an increase in body fat but it is unclear whether there is a causal relationship (Costa et al., 2017; Elkafrawy et al., 2021).

- Based on randomized controlled trials, VKM (2015) concluded that doses up to 1200 mg NAC per day (corresponding to 900 mg per day, or approximately 13 mg/kg bw per day, cysteine or cystine) and in some studies up to 2400 mg NAC (corresponding to 26 mg/kg bw per day L-cysteine or L-cystine) did not lead to adverse effects. They concluded that a dose of 13 mg/kg bw per day L-cysteine or L-cystine would be unlikely to cause health effects in individuals of 10 years and older (assuming similar tolerance as in adults). Studies that were published since did not alter this conclusion. In general, there were no statistically significantly more adverse events reported after the use of NAC (daily dose range: 1000 – 6000 mg) compared to placebo. The most commonly reported side effects included gastrointestinal symptoms, like nausea. It must be noted however that most studies were conducted in patient groups, i.e. persons that may be more prone to the development of adverse events.

Toxicological reference value

VKM (2015) used a dose of 900 mg per day for L-cysteine and L-cystine, corresponding to 13 mg/kg bw per day for a 70-kg adult, as value for comparison in the risk characterization. This was based on the absence of adverse effects at a daily dose of 1200 mg NAC in human studies in various population groups (VKM, 2015). Assuming similar tolerance for children (10 – <14 years) and adolescents (>14 – 18 years), VKM also used the value of 13 mg/kg bw per day for the risk characterization for these age groups.
The information in human studies published since the VKM opinion does not give cause to alter the conclusion that 13 mg/kg bw per day L-cysteine or L-cystine is a safe dose for adults, adolescents and children (10 – <14 years). Hence, in the current assessment this value of VKM will be used as toxicological reference value for the risk assessment.

It is noted that this value exceeds the therapeutic dose for NAC of 600 mg per day, corresponding to 450 mg L-cysteine[5] or 6.4 mg/kg bw for a 70-kg adult. However, we considered the therapeutic effect of NAC (mucolytic agent) not adverse.

For children of 2 – 10 years, the therapeutic dose for NAC will lead to higher exposures to L-cysteine than the value of VKM of 13 mg/kg bw per day. Therefore, it is considered justified to use the value of VKM for children of these ages as well.

For children younger than 2 years, the use of NAC is contraindicated (see also section 6.3). Therefore, for this age group no safe dose or reference value can be determined.

In Table 1, the toxicological reference value is expressed in mg/day for L-cysteine and L-cystine and NAC for the different age groups.

[5] Or L-cystine

Table 1 Toxicological reference values for L-cysteine and L-cystine and NAC for different age groups

Age group (years)	Average body weight (kg)	Reference value L-cysteine and L-cystine (mg/kg bw per day)	Reference value L-cysteine and L-cystine (mg/day)	Reference value NAC (mg/day)
>18	70[a]	13	900	1200
11 – 18	44.8[b]	13	580	770
6 – 11	24.3[b]	13	320	430
2 – 6	14.3[b]	13	190	250
0 – 2 years	NA[c]	NA[c]	NA[c]	NA[c]

[a] Default body weight for adults used by VKM (2015).
[b] Default values for body weight of children aged, respectively, 11-16, 6-11 and 2-6 years reported by Te Biesebeek et al. (2014).
[c] Not applicable. For this age group, the use of NAC as a therapeutic agent is contraindicated and therefore no reference value could be derived.

Risk assessment

The daily exposure to NAC or L-cysteine from the use of food supplements varies depending on the food supplement used. The estimated exposure to NAC ranged between 500 to 2000 mg per day corresponding to 7.1 – 29 mg/kg bw per day for an adult of 70 kg when food supplements containing NAC are used. This yields an exposure to L-cysteine of approximately 375 to 1500 mg[6], corresponding to 5.4 – 21 mg/kg bw per day for an adult of 70 kg. When food supplements containing L-cysteine are used the exposure to L-cysteine amounts to 500 to 1500 mg per day corresponding to 7.1 – 21 mg/kg bw per day. For adults the use of part of the food supplements containing NAC and/or L-cysteine leads to an exposure above the reference value of 13 mg/kg bw per day. Accordingly, in these cases the development of gastrointestinal complaints or other side effects as mentioned for medicinal products cannot be ruled out.

The same holds for children of 2 years and older. For this age group, exceedance of the 13 mg/kg bw per day will occur for more food supplements since, due to their lower body weights, the relative exposure per kg body weight will be higher.

For children under 2 years of age the use of NAC is contraindicated due to their inability to cough up mucus. Therefore, the use of food supplements containing NAC, L-cysteine or L-cystine for this age group is considered not safe.

Besides exposure resulting from food supplement use, relatively low exposure to L-cysteine occurs via food because it is a component of food proteins and it is authorized for use as a food additive and flavouring agent.

[6] Calculated based on molecular weights (see section 3.1)

In addition, when NAC is also used as a mucolytic agent (medicine) an additional exposure to L-cysteine of 450 mg (for children > 7 years and adults) or 300 mg (children aged 2-7 years) may take place (see section 4.2). Therefore, when food supplements and medicines containing NAC and/or L-cysteine or L-cystine are combined there is a higher risk for the development of gastrointestinal complaints or other side effects.

Conclusion and recommendations

In 2015, VKM determined a reference value for L-cysteine of 13 mg/kg bw per day, that is considered to not lead to appreciable health risks. The review of recent human studies does not warrant an adjustment of this value.

This reference value equals doses of 900 mg/day L-cysteine or L-cystine corresponding to 1200 mg/day NAC for adults. For children aged 11 – 18 years this amounts to 580 mg/day L-cysteine or L-cystine and 770 mg/day NAC. For children aged 6 – 11 years, this equals 320 mg/day L-cysteine or L-cystine and 430 mg/day NAC, and for children aged 2 – 6 years 190 mg/day L-cysteine or L-cystine and 250 mg/day NAC. Food supplements with a recommended daily dose below these reference values are therefore considered to not lead to health risks.

For children under 2 years of age the use of NAC as a medicine is contraindicated and therefore, the use of food supplements containing NAC, L-cysteine or L-cystine is considered not safe for this age group.

When comparing the estimated exposure based on recommended use to the reference value, part of the food supplements available on the Dutch market leads to an exposure to L-cysteine that is higher than 13 mg/kg bw per day. This means that in these cases the development of gastrointestinal complaints or other side effects as mentioned for medicinal products cannot be ruled out.

It is not recommended to concomitantly use medicines containing NAC and food supplements containing NAC, L-cysteine and/or L-cystine, since this will lead to exceeding the reference value of 13 mg/kg bw per day.

1 Introduction

1.1 Background

In December 2020, the Minister for Medical Care and Sport of the Ministry of Health, Welfare and Sport (VWS) announced the actions that would be taken to better regulate food supplements and herbal preparations in the Netherlands, thereby facilitating enforcement. One of those actions is to expand the list included in the Herbal Preparations Decree of the Dutch Commodities Act[7] with substances/botanicals that are either forbidden or restricted (i.e. subject to a maximum level) in food supplements or herbal preparations (Van Ark, 2020). In order to determine whether a substance or botanical needs to be included in this list, a risk assessment is warranted. The selection of substances and botanicals chosen for risk assessment was based on the prerequisites that the substances/botanicals were sold on the Dutch market and (widely) used in the Netherlands and there were indications for possible health risks, e.g. from Rapid Alert System for Food and Feed (RASFF) reports or from enforcement institutes. The current assessment is about food supplements containing N-acetyl-L-cysteine (NAC).

NAC is the N-acetyl derivative and precursor of the endogenous amino acid L-cysteine. In the body, NAC is metabolized to L-cysteine, and L-cysteine can be interconverted to/from its dimer L-cystine (EFSA, 2003; VKM, 2015). NAC is also used in medicinal products registered in the Netherlands (Farmacotherapeutisch Kompas, 2022a, b, c). Food supplements containing NAC are sold on the Dutch market to supply extra cysteine to the body for production of glutathione. Glutathione is an antioxidant and is involved in the detoxication of substances in the body. Since NAC is metabolized to L-cysteine and L-cystine, information on these compounds is included in this assessment as well.

1.2 Information on existing assessments

1.2.1 European Food Safety Authority (EFSA)

EFSA evaluated the use of NAC in foods for particular nutritional uses and in foods for special medical purposes (FSMPs; EFSA, 2003). EFSA concluded that the proposed use of NAC for replacement of L-cysteine in foods for particular nutritional uses is not acceptable, because the proposed uses would result in intakes of up to 2900 mg per day of NAC exceeding the therapeutic dose of 400 to 600 mg per day, which have mucolytic effects. In addition, gastrointestinal complaints such as vomiting and diarrhoea could not be excluded in sensitive individuals. Further, there was some concern because of a potential prooxidative effect of NAC at a dose of 1200 mg but the clinical relevance is unknown. On the other hand, it was concluded that the inclusion of NAC in the list of substances permitted in FSMPs is acceptable, given that FSMPs should be used under the supervision of a physician (EFSA, 2003).

[7] https://wetten.overheid.nl/BWBR0012174/2020-07-01. Accessed 8 July 2022.

EFSA has also evaluated the use of L-cysteine as a flour treatment agent in biscuits intended for infants and young children (see also subheading Scientific Committee on Food for other uses). The estimated intake is considered to be very low in comparison with the intake provided by the remainder of the child's diet and EFSA concluded that this application is of no safety concern (EFSA, 2006).

Further, EFSA evaluated the implications for human health of using L-cystine and L-cysteine hydrochloride as flavouring agents in or on foodstuffs. EFSA concluded that the exposure to L-cystine and L-cysteine through their natural occurrence in food is orders of magnitude higher than the anticipated level of exposure from the use of these substances as flavouring substances (23 and 73 mg/person per day for L-cystine and L-cysteine, respectively, based on the modified Theoretical Added Maximum Daily Intake (mTAMDI) approach). The substances were concluded not to be of safety concern at their estimated levels of dietary exposure as flavouring substances (EFSA, 2007).

1.2.2 Scientific Committee on Food (SCF)

SCF evaluated the use of L-cysteine (as hydrochloride or monohydrate) as a food additive. SCF considered its use as a flour treatment agent toxicologically acceptable. L-cysteine is a non-essential amino acid, occurring in a wide variety of foods, especially cereals, and the contribution to the total daily dietary intake from the use in bakery processes was considered insignificant (SCF, 1991).

1.2.3 Joint FAO/WHO Expert Committee on Food Additives (JECFA)

JECFA evaluated the use of L-cysteine as a flavouring agent. JECFA concluded that because L-cysteine is a macronutrient and a normal component of proteins, human exposure through food is orders of magnitude higher than the anticipated level of exposure from use as a flavouring agent. No safety concerns would therefore raise at the estimated intake of L-cysteine when used as a flavouring agent (JECFA, 2004).

1.2.4 Institute of medicine (IOM)

In 2005, the IOM of the National Academy of Sciences of the United States published a report on several nutrients, including L-cysteine and L-cystine. They concluded that the available data on the adverse effects of L-cysteine and L-cystine resulting from the use of supplements are not adequate for a dose-response assessment and the derivation of a tolerable upper intake level. They mentioned that single oral doses of 5 and 10 g of L-cysteine have been reported to produce nausea and light-headedness (IOM, 2005).

1.2.5 French Agency for Food, Environmental and Occupational Health & Safety (ANSES)

In 2011, ANSES published an assessment of the risks associated with substances with nutritional or physiological effects, including cysteine. They listed reported adverse effects of cysteine, including hypercholesterolemia, fatty liver and neurotoxicity. ANSES concluded that a proper risk assessment for amino acids, including cysteine, could not be conducted due to the complexity of amino acid metabolism, the

scarcity of toxicological data, and the insufficiently characterized risks (ANSES, 2011).

1.2.6 Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN)/Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN))

In 2012, the Scientific Committee of AESAN assessed 49 substances, including the amino acid L-cysteine, for their use in food supplements (AESAN, 2012). The Scientific Committee of AESAN concluded that the proposed maximum daily intake of 300 mg L-cysteine is "lower than the requirements of L-methionine + L-cysteine established by the World Health Organization (WHO) and is very far from the doses of L-cysteine that cause dizziness and nausea". Therefore, the Scientific Committee considered it acceptable in terms of safety for use as a food supplement. In 2015, the Scientific Committee of AECOSAN assessed a maximum daily intake of 300 mg NAC from food supplements (AECOSAN, 2015). This proposal was based on the fact that higher doses of NAC are considered to be medicinal in Spain. Based on the toxicological information available, AECOSAN concluded that a maximum daily amount of 300 mg NAC is acceptable from a safety point of view.

1.2.7 Norwegian Scientific Committee for Food Safety (VKM)

In 2015, VKM assessed the safety of the use of food supplements providing L-cysteine in a daily dose of 10 mg or L-cystine in a daily dose of 250, 500, 750 and 1000 mg (VKM, 2015). VKM included studies with NAC in its assessment. Since NAC is also used as a human medicine, mainly as a mucolytic agent, data from clinical trials studying mostly doses of about 600-1200 mg per day were available. VKM concluded that a dose of 1200 mg NAC per day, which yields doses of L-cysteine or L-cystine up to 900 mg per day corresponding to 13 mg/kg bw per day for a 70 kg adult, does not lead to appreciable health risks in adults. The data for NAC doses above 900 mg per day are scarce. There were no indications that children ≥ 10 years of age and adolescents are more vulnerable (on a g/kg bw basis) than adults for these compounds. Based on this information, VKM concluded that the proposed doses of 10 mg per day L-cysteine and doses of 250, 500 and 750 mg per day L-cystine are considered to be unlikely to cause adverse health effects in adolescents (14 to 18 years) and adults (≥18 years), whereas the proposed dose of 1000 mg per day L-cystine may represent a risk of adverse health effects. For children (10 to 14 years) doses of 10 mg per day L-cysteine and doses of 250 and 500 mg per day L-cystine were considered to be unlikely to cause adverse health effects, whereas the doses of 750 and 1000 mg per day L-cystine may represent a risk of adverse health effects. Children below 10 years of age were outside the terms of reference for the risk assessment.

1.2.8 Swiss Federal Food Safety and Veterinary Office (FSVO)

In 2016, FSVO assessed the safety of a specific food supplement containing a daily dose of 50 mg per day NAC intended for use by women of childbearing age. The assessment (unpublished) concluded that there were no toxicological concerns for the proposed daily dose of 50 mg NAC since this dose is 8-12 times lower than the therapeutic mucolytic dose of NAC in oral medicines, that are also given to pregnant women (FSVO, 2016).

1.3 Information on existing legislations

Currently, in the Netherlands, there are no specific restrictions for the use of NAC, L-cystine or L-cysteine in food supplements and/or herbal preparations[8].

There are several medicinal products with (natrium)acetylcysteine as active substance registered in the Netherlands. These medicines are used orally as mucolytic agents, intravenously as paracetamol antidote and as eyedrops for treatment of keratoconjunctivitis sicca [9,10] (Farmacotherapeutisch Kompas, 2022a, b, c).

In Europe, L-cysteine is listed as a food additive (E 920) for use at *quantum satis* in flour and at a maximum of 1000 mg/kg in biscuits for infants and young children (Regulation (EC) No 1333/2008). In addition, L-cysteine may be present as food additive in food enzymes at maximum levels of 10,000 mg/kg in the enzyme preparation, 10 mg/kg in final food except beverages and 5 mg/L in beverages (EC, 2008a). L-cystine and L-cysteine are both authorized as flavouring agents (Regulation (EC) No 1334/2008).

In many EU Member States NAC and L-cysteine have a so-called dual status meaning that it is present on the market as both a medicinal product and a food supplement. This means that factors like the presentation of the product and dosage may determine which regulation applies to the product.

1.4 Current risk assessment

The current risk assessment uses the most recent and comprehensive assessment of VKM as starting point supplemented with literature data that has been published since. Hence, the literature search is limited to an update of the search as performed by VKM.

[8] wetten.nl - Regeling - Warenwetbesluit Kruidenpreparaten - BWBR0012174 (overheid.nl) Accessed July 2023.
[9] Dry eyes due to decreased tear production
[10] Home | Geneesmiddeleninformatiebank | College ter Beoordeling van Geneesmiddelen. Accessed July 2023

2 Literature search

First, assessments performed by other institutes were searched for and shortly summarized. As stated, the assessment of VKM (2015) was then selected as starting point for the current risk assessment and an update of their literature search was performed. Literature that has been published in the period 2015 – 2023 was searched in Pubmed using the search strategy as presented in Annex I (based on the VKM search strategy). In addition, reference lists of the obtained meta-analyses were checked to identify any missing studies. In total, 16 additional human studies were included in the current report as well as eight meta-analyses.
VKM did not include animal studies in their literature search due to the fact that numerous human studies were available and animal studies were included in the previous risk assessments. However, since an effect of cysteine on cholesterol in animals was described by ANSES (2011), VKM additionally searched Pubmed using the search terms 'cholesterol OR lipid OR lipoprotein AND cysteine or cysteine' to identify studies on cholesterol (performed on 26 May 2015). To update this search in Pubmed, the same search terms were used in the current assessment for the time period 2015 – 2023. A total of 295 references were obtained of which six were considered relevant and included in the current report.

3 Description of the product

3.1 Identity and nature of the source material

3.1.1 *Substance*

NAC (IUPAC name N-acetyl-L-cysteine; CAS number 616-91-1; MW 163.2 g/mol[11]), commonly known as acetylcysteine, can be found in plants of the *Allium* species, especially in the onion (*Allium cepa*, 45 mg/kg NAC) (Šalamon et al., 2019). NAC can also be manufactured synthetically (EFSA, 2003).

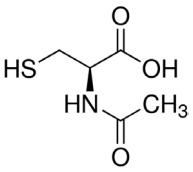

Figure 1 Molecular structure of NAC (AECOSAN, 2015).

NAC is a N-acetyl derivative and a precursor of the endogenous amino acid L-cysteine (MW 121.16 g/mol[12]). The acetyl group in NAC acts merely as a molecule stabiliser (VKM, 2015), it can protect the amino group of the parent amino acid from chemical interactions (EFSA, 2003). In the human body, NAC can be deacetylated to L-cysteine. L-cysteine can be converted to L-cystine (the dimer of L-cysteine, MW 240.3 g/mol) via an oxidation step, and vice versa, L-cystine can also be converted to L-cysteine via a reduction step (Figure 2). Thus, one L-cystine molecule is reduced to two L-cysteine molecules.

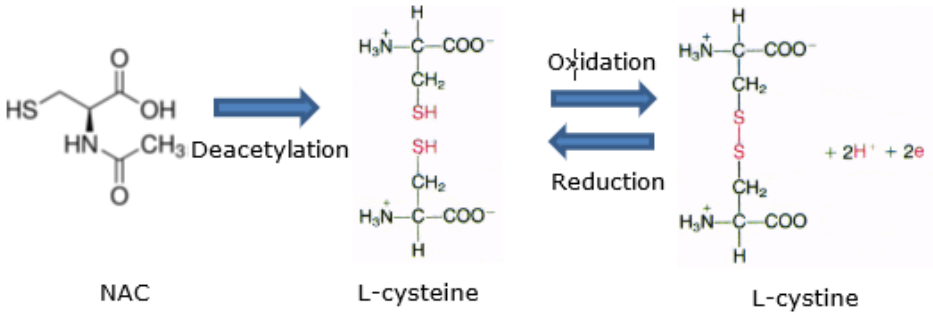

Figure 2 Relationship between NAC and L-cysteine and L-cystine (based on VKM (2015).

3.2 Manufacturing process

NAC can be manufactured by acylating hydrochloride monohydrate with acetic anhydride (the acylating agent) in an alkaline aqueous medium (EFSA, 2003).

3.3 Selected compound(s) for risk assessment

Because NAC is metabolized to L-cysteine in the human body, and L-cysteine can be interconverted to/from its dimer L-cystine, the selected

[11] Acetylcysteine | C5H9NO3S | CID 12035 - PubChem (nih.gov)
[12] Cysteine | C3H7NO2S | CID 5862 - PubChem (nih.gov)

compounds for the current risk assessment are N-acetylcysteine, cysteine and cystine.

3.4 Stability

NAC is more stable than its metabolite L-cysteine (EFSA, 2003), because the N-acetyl group is acting as a molecule stabilizer (VKM, 2015).

3.5 Use and use levels

Various food supplements containing NAC, L-cysteine, L-cystine or a combination can be found on the Dutch market mainly as a capsule. Examples of these food supplements are presented in Table 2.
The recommended use generally varies from 1 to 3 capsules per day resulting in recommended daily doses ranging from 200 to 2000 mg NAC, L-cysteine or L-cystine. For some food supplements warning phrases are used, such as to consult an expert in case of illness, medication use, pregnancy and breastfeeding. In one case it was specifically mentioned that the food supplement is suitable for children of 3 years and older. And also in one case it was mentioned to not use the food supplement in combination with protein-containing foods.

In addition, NAC is also used as a human medicine due to its pharmacological activity. Currently, there are 50 medicinal products (including different dose strengths of the same brand) registered at the Medicines Evaluation Board (MEB, 2023). An overview of the doses used and resulting exposure is given in chapter 4.

Table 2 Examples of food supplements containing NAC, L-cysteine, L-cystine or a combination available on the Dutch market

Number	Ingredient(s)	Dose per serving	Recommended daily use	Maximum recommended daily dose
1	N-acetyl-L-cysteine	600 mg	1 to 2 capsules	1200 mg
2	N-acetyl-L-cysteine	500 mg	1-2 times 1-2 capsules	2000 mg
3	N-acetyl-L-cysteine	600 mg	1 to 2 capsules	1200 mg
4	N-acetyl-L-cysteine	600 mg	1 capsule	600 mg
5	N-acetyl-L-cysteine	600 mg	1 to 2 capsules	1200 mg
6	N-acetyl-L-cysteine	500 mg	1 capsule	500 mg
7	N-acetyl-L-cysteine, reduced L-glutathione, L-cysteine, selenium-L-methionine	500 mg (NAC) 25 mg (L-cysteine)	3 times 1 capsule	1500 mg (NAC) 75 mg (L-cysteine)
8	N-acetyl-L-cysteine, zinc citrate, BioPerine® (Piper nigrum L. extract), retinyl acetate	600 mg	1-2 capsules	1200 mg
9	L-cysteine	600 mg	1-2 capsules	1200 mg
10	L-cysteine	500 mg	2-3 times 1 capsule	1500 mg
11	L-cysteine	500 mg	1 capsule	500 mg
12	L-cystine, L-taurine, L-lysine, L-arginine, zinc pidolate, opti-MSM (methylsulfonylmethane), calcium ascorbate, equisetum arvense foliae extract 3-5/1, urtica dioicabio extract 7-8/1, inositol, selenium, vitamins	200 mg	1 capsule	200 mg
13	L-cystine, keratine, blueberry extract, vitamins, copper	200 mg	2 tablets	400 mg

4 Exposure: extent and duration

4.1 Exposure from food supplement use

Based on their recommended use, the exposure to NAC resulting from the use of food supplements containing NAC will vary from 500 to 2000 mg per day (Table 2). For an adult of 70 kg this amounts to an exposure to NAC of 7.1 – 29 mg/kg bw per day. This yields an exposure to L-cysteine of approximately 375 to 1500 mg[13], corresponding to 5.4 – 21 mg/kg bw per day for an adult of 70 kg. In addition, there are food supplements available that contain L-cysteine itself. When using the examples presented in Table 2 this results in an exposure of 500 to 1500 mg L-cysteine per day, corresponding to an exposure of 7.1 – 21 mg/kg bw per day for a 70-kg adult. One supplement contained both NAC and L-cysteine resulting in a total calculated daily exposure to L-cysteine of 1200 mg.
Further, some food supplements contain L-cystine at a recommended daily dose of 200 to 400 mg, corresponding to an exposure of 2.9 – 5.7 mg/kg bw per day for a 70-kg adult.

As only in some cases use by children is advised against, exposure to NAC, L-cysteine and L-cystine by children due to the use of food supplements may occur in similar absolute amounts as for adults. Due to their lower bodyweights, the relative exposure per kg bodyweight will however be higher.

4.2 Possibility of additional/combined human exposure

4.2.1 Medicinal products

NAC is also used as a mucolytic agent or to treat a paracetamol overdose or eye disease. In the Netherlands, several human medicines are registered and available on the Dutch market. Additional exposure may therefore occur when people use NAC as medicine together with (multi-ingredient) supplements containing NAC. Table 3 shows an overview of the different medical uses of NAC and the associated doses.

Combined use of food supplements and the medicinal product for the treatment of a paracetamol overdose is considered an exceptional case and therefore not taken further into account.
Based on the use information, the use of NAC as mucolytic agent may result in an additional exposure of 400 mg per day for children up to 7 years and of 600 mg per day for children >7 years and adults. For a 70-kg adult this means an additional exposure of 450 mg L-cysteine corresponding to 6.4 mg/kg bw per day.

[13] Calculated based on molecular weights (see section 3.1)

Table 3 Overview of different medical uses of NAC (Farmacotherapeutisch Kompas, 2022a, b, c)

Medical purpose	Route	Dose and use per age group
Mucolytic agent	oral	• Children 2-7 years: 400 mg taken as 2 times a day 200 mg • Children 7-18 years: 600 mg taken as 3 times a day 200 mg • Adults >18 years: 600 mg taken either in one time or 3 times a day 200 mg • For children <2 years, the use of NAC is contraindicated.
Paracetamol antidote	IV	• Starting dose: 150 mg/kg bw in 200 mL • Follow-up: 50 mg/kg bw in 500 mL for 8 hours or 75 mg/kg bw every 4 hours
Keratoconjunctivitis sicca[a] treatment	eyedrops	1 drop (50 mg/mL) 3-4 times a day for children and adults

[a] Dry eyes due to decreased tear production

Additional exposure may also occur when NAC is used as eyedrops for treatment of keratoconjunctivitis sicca. The volume of one drop is normally reported to be in the range of 25 to 70 µL (Van Santvliet & Ludwig, 2004), and the concentration of NAC in eyedrop is 50 mg/ml (Farmacotherapeutisch kompas, 2022b). Assuming as a worst case scenario a volume of 70 µL per drop, one drop 4 times a day and a NAC concentration of 50 mg/mL, the additional exposure can be estimated to be 14 mg NAC per day for both children and adults. This is considered a negligible amount when compared to the exposure resulting from food supplement use.

4.2.2 Food improvement agents

The estimated dietary exposure of cystine and L-cysteine hydrochloride from the use as flavouring agents was 23 and 73 mg/person per day respectively, based on the mTAMDI approach (EFSA, 2007).
No estimated dietary exposure via the use of food additives was found.

4.2.3 Dietary exposure

L-cystine and L-cysteine are present in the normal diet as components of food proteins, mainly cereal proteins and animal proteins (VKM, 2015). The mean daily intake of cysteine in all population groups in the USA was 1.0 g per day, based on data from the 1988-1994 National Health and Nutrition Examination Survey (NHANES III) (IOM, 2005).
No data for the European population has been identified.

4.2.4 Endogenous

L-cysteine could be formed endogenously from L-serine and essential amino acid L-methionine (IOM, 2005).

4.3 Information on historical use of the ingredient

NAC is patented in 1960, and its use in medicine was first reported in 1967 (Schwalfenberg, 2021).

5 Biological data

This chapter contains a brief summary of the toxicokinetic and toxicological data and the main conclusions as drawn in previous assessments of VKM and EFSA supplemented with a short description of the new identified studies from the updated literature search of VKM (2015).

5.1 Toxicokinetics

5.1.1 Absorption, distribution, metabolism, excretion

Following oral exposure, NAC is rapidly absorbed in humans with the maximum plasma concentration of NAC being obtained around 1-2 hours (Holdiness, 1991). The oral bioavailability of NAC is around 6-10% (Borgström, Kagedal & Paulsen, 1986). The intake of NAC results in increasing plasma levels of L-cysteine or L-cystine, non-protein and protein SH groups, and disulphide-bound thiols, indicating that NAC is metabolized to cysteine and further metabolites (including incorporation into proteins) (Maddock, 1980). The terminal half-life of NAC is 6.25 hours following oral intake of 400 mg (Olssen et al., 1988). Besides extensive metabolism takes elimination also place via renal clearance. It was found that about 3% was excreted in faeces (Bonanomi & Gazzaniga, 1980). Another study reported that approximately 22% of radioactivity is excreted unchanged in urine within 24 hours when 100 mg radiolabelled NAC was orally administered to patients with respiratory disorders (Rodenstein, Coster & Gazzaniga, 1978). In another study 3.66% and 3.80% was excreted unchanged in urine after oral administration of 600 mg NAC in Chinese and Caucasian healthy subjects, respectively (Papi, Di Stefano & Radicioni, 2021). In patients with end-stage renal disease or severe liver injury the kinetics of NAC is altered due to diminished total clearance (Jones et al., 1997; Nolin et al., 2010).

5.2 Toxicological data

5.2.1 Acute toxicity

In the studies evaluated by EFSA the median oral lethal dose (LD_{50}) values for NAC in mice and rats were greater than 6000 mg/kg bw (Bonanomi & Gazzaniga, 1980).

In the studies evaluated by VKM the oral LD_{50} values for L-cysteine in mice were 4200 mg/kg bw for females and 3550 mg/kg bw for males (Takasaki et al., 1973). In rat, the oral LD_{50} values for L-cysteine were 5580 mg/kg bw for females and 6350 mg/kg bw or 1890 mg/kg bw (gavage) for males (Takasaki et al., 1973; Sprince et al., 1974).

For L-cystine, the oral LD_{50} value in both male and female rat was >25,000 mg/kg bw (Kawai et al., 1978).

5.2.2 Short-term and sub-chronic toxicity

After repeated oral administration of NAC to male and female rats in doses up to 2000 mg/kg bw per day for 4 weeks and in doses up to 1000 mg/kg bw per day for 12 and 28 weeks, no effects were observed

on behaviour, body weight gain, haematology, hepatic and renal function, prothrombin and bleeding time. Also, histological examination and necropsy findings did not show pathological lesions (Bonanomi & Gazzaniga, 1980).

After oral administration of L-cysteine to mice (sex not reported; n=8-12 per dose group) at doses of 0, 200, 1000 or 3000 mg/kg bw per day and rats (sex not reported; n=9-12 per dose group) at doses of 0, 200, 1000 or 5000 mg/kg bw per day for 30 days, or to male rats (n=10-12 per dose group) at doses of 0, 100, 500 or 2000 mg/kg bw per day for 6 months, adverse effects were observed at all treatment doses [no further information] (Takasaki et al., 1973).

A NOAEL of 600 mg/kg bw was identified after daily oral administration of 0, 100, 300, 600 or 3000 mg/kg bw cystine to male and female rats (n=14 or 20 per group) for 93 days (Kawai et al., 1978). No further details were provided.

5.2.3 Genotoxicity

No mutagenic effect of NAC was observed in an Ames test using *Salmonella typhimurium* strains (not further specified) with and without metabolic activation using hepatic microsomes (Bonanomi & Gazzaniga, 1980).

No effects of L-cysteine were observed in a chromosomal aberration assay using Chinese hamster ovary cells at a concentration of 61 µg/ml (Stich et al., 1981). EFSA (2007) stated there was no detailed description of the experimental system available and therefore the validity of the study could not be evaluated. Further, no effects were observed in a sister chromatid exchange assay using V79 Chinese hamster ovary cells at two concentrations (max. 121 µg/ml with and without S9 mix (Speit et al., 1980). EFSA (2007) concluded that this study is of limited relevance since the isomer was not specified and cytotoxicity was not measured.

L-cystine had a negative result in a bacterial reverse mutation assay (plate method) using *E. coli uvrB* or *uvrB umu C* or *uvrB LexA* at a concentration of 481 µg/ml (2mM) without S9 mix (Sargentini & Smith, 1986). EFSA (2007) concluded that this study was a valid non GLP-study, although it was conducted in the absence of a metabolic activation system.

5.2.4 Chronic toxicity and carcinogenicity

After repeated oral administration of NAC to male and female dogs up to 300 mg/kg bw per day for 52 weeks, no effects were observed on behaviour, body weight gain, haematology, hepatic and renal function, prothrombin and bleeding time. Also, histological examination and necropsy findings did not show pathological lesions (Bonanomi & Gazzaniga, 1980).

No chronic toxicity studies with L-cysteine or L-cystine and no carcinogenicity studies with NAC, L-cysteine or L-cystine were reported by EFSA (2003) or VKM (2015).

5.2.5 *Reproduction and developmental toxicity*

In a fertility study, administration of NAC to male rats (n=12) at doses of 0, 250, 500 and 1000 mg/kg bw per day 15 weeks before pairing and during the mating period resulted in no adverse effects at a dose of 250 mg/kg bw per day. At the higher doses though a slight reduction of fertility was observed (Bonanomi & Gazzaniga, 1980).

In a developmental toxicity study, administration of NAC to rats (n=25) at doses of 0, 500, 1000 and 2000 mg/kg bw per day from day 6 to 15 of pregnancy did not show any developmental effects. Also in a similar experiment with rabbits, no adverse effects were observed (Bonanomi & Gazzaniga, 1980).

After dosing rats (n=20) with 0, 250, 500 or 1000 mg/kg bw per day NAC from day 15 of pregnancy through day 21 post-partum, no adverse effects on delivery and lactation or on physical development and maturation of the offspring were observed (Bonanomi & Gazzaniga, 1980).

In a six generation reproduction study in rats, a NOAEL of 3500 mg/kg L-cysteine in diet (equivalent to 175 mg/kg bw per day) was derived (the only dose tested) (Frape et al., 1971).

5.2.6 *Effects on cholesterol*

VKM (2015) noted in their assessment that according to the assessment of ANSES (2011), high doses of cysteine can result in fatty liver and hypercholesterolemia as observed in animal studies. The two additional studies identified by VKM on the effect on cholesterol did not support this observation (Korou et al., 2014; Lin and Yin, 2008). The updated literature search for the current assessment identified three additional animal studies (Niewiadomski et al., 2016; Liou et al., 2021; Hang et al., 2021) and these are summarized below.

Niewiadomski et al. (2016) fed cysteine dioxygenase (Cdo1)-null and wildtype C57BL/6 mice a basal diet, a high-fat diet or a high-fat, taurine-supplemented diet for approximately 25 days. A lack of cysteine dioxygenase leads to elevated tissue and plasma cysteine levels. When the Cdo1-null mice received a high fat diet, they gained more weight than the wildtype control mice. They also had higher feed intakes, lower leptin levels and higher abundance of hepatic stearoyl-CoA desaturase 1 compared to the control mice.

Male C57B1/6 mice were fed a normal chow diet or a high-fat diet either with (starting 1 week prior to diet) or without 150 mg/kg bw NAC by gavage for 8 weeks (Hang et al., 2021). In addition, one group was first fed a normal diet or high-fat diet for 8 weeks whereafter they were gavaged with 150 mg/kg bw NAC for another 4 weeks. Blood samples were collected every 2 weeks for blood chemistry and liver tissues were obtained after the treatment period for further investigation. Less hepatic triglyceride accumulation and lipid droplet formation was observed in mice fed a high-fat diet + NAC compared to fed a high-fat diet only. The authors concluded that NAC may be effective in treating nonalcoholic fatty liver disease.

Liou et al. (2021) studied the toxicological mechanisms of NAC overdose of 275, 400 or 800 mg/kg bw via intraperitoneal injection in BALB/c mice. Survival rates were reduced at 48 hours to 90% in the 400 mg/kg bw group and to 70% in the 800 mg/kg bw group. Body weight was statistically significantly reduced in all dose groups compared to control animals. The authors observed that serum ALT and AST activities statistically significantly increased with increasing doses at all dose groups at 24 hours, and returned to levels similar to the control group at 48 hours in the 275 and 400 mg/kg bw groups but not in the 800 mg/kg bw group. In addition, in the 800 mg/kg bw group, serum nitrogen and creatinine levels statistically significantly increased at 24 hours but decreased to normal levels thereafter, and hepatic gluthatione levels statistically significantly decreased at both 24 hours and 48 hours after injection. Also serum levels of inflammatory cytokines, like IL-6, increased at high doses of NAC, especially after a dose of 800 mg/kg bw. In both the 400 and 800 mg/kg bw groups, lipid accumulation as well as dehydration were observed in the liver at 48 hours. Hepatic triglyceride levels were statistically significantly increased in all dose groups 48 hours post-dose, while serum triglyceride statistically significantly decreased. Total cholesterol levels were statistically significantly increased in the highest dose group. The authors conclude that NAC overdose induces hepatic and systemic inflammations and interferes with fatty acid metabolism.

Taken together, these studies seem to suggest that high doses of NAC, approximately a factor of 10 higher than human doses, see section 4.1, may interfere with the fatty acid metabolism and lead to hepatotoxicity while at low levels this negative effect is not observed.

5.2.7 Human data

VKM (2015) performed a literature search using the databases Medline, Embase and Global Health (publication dates up till 5 March 2015) for human studies looking at adverse effects caused by cysteine and cystine. Because there were only a few studies with L-cysteine or L-cystine, studies with NAC were also included. In addition, they included previous risk assessments on L-cysteine from IOM (2005), EFSA (2006, 2007), ANSES (2011), AESAN (2012) and on NAC from EFSA (2003).

Orally administered doses of 5 and 10 g of L-cysteine caused nausea and slight dizziness. Also, various adverse effects (e.g. fatigue, dizziness, nausea and insomnia) were observed in healthy people who were administered increasing doses of up to 20 g of L-cysteine (with the non-selective monoamino-oxidase (MAO) inhibitor tranylcypromine) (VKM, 2015).

Based on the identified human studies, VKM concluded that there were no differences in severe adverse events between the placebo and NAC-groups in the randomized control trials. Further they concluded that the included studies conducted in adults showed that doses up to 1200 mg NAC per day (corresponding to 900 mg per day cysteine or cystine), and in some studies up to 2400 mg per day (corresponding to 1800 mg for cysteine or cystine), did not lead to adverse effects. Hence, they concluded that a dose of 13 mg/kg bw per day cysteine or cystine, based on a dose of 900 mg per day for a 70-kg adult, would be unlikely

to cause health effects. For children (10 to <14 years) and adolescents (14 to <18 years), they concluded that only a few studies dosing up to 500 mg per day for a short duration were available. However, since there are no data indicating that children aged 10 years or older are more vulnerable for cysteine or cystine, a similar tolerance was assumed and doses up to 13 mg/kg bw per day were considered unlikely to cause health effects. Children below 10 years of age were outside the terms of reference for their risk assessment (VKM, 2015).

Since the assessment of VKM included human studies (randomized control trials; RCTs) up to March 2015, additional studies in humans looking at adverse effects of NAC, cysteine or cystine that were published more recently are presented in Table 4. In general, there were no statistically significantly increases in adverse events reported after the use of NAC (daily dose range: 1000 – 6000 mg) compared to placebo. Out of the in total 14 additionally identified human studies in adults, only one study reported statistically significantly more gastrointestinal symptoms in NAC-treated individuals compared with the control group (Berk et al., 2019). In this study the daily dose was 2000 mg NAC (yielding 1500 mg L-cysteine corresponding to 21 mg/kg bw for an adult of 70 kg). It must be noted however that most studies were conducted in patient groups, i.e. persons that may be more prone to the development of adverse events. Hence, it is difficult to know whether similar observations would be made in the healthy population. In addition, also the two additionally identified studies in children/adolescents (8 – 17 years) did not show statistically significant differences in adverse events in NAC-treated individuals compared with the control group at doses of 2400 – 2700 mg per day.

Additionally, several meta-analyses were identified that commented on the occurrence of adverse events after NAC treatment. The most commonly reported side effects included gastrointestinal symptoms, like nausea. In almost all cases it was concluded that NAC did not statistically significantly increase the risk of adverse events compared with placebo nor was the occurrence of side effects dose-dependent; however at high doses of NAC (at least >1 gram per day) higher rates compared to placebo of gastrointestinal effects were observed (Cazzola et al., 2015; Rogliani et al., 2016; Fowdar et al., 2017; Feng et al., 2019; Calverley, Rogliani & Papi, 2021; Kishi et al., 2020; Kishi, Sakuma & Iwata, 2022; Parli, Gales & Gales, 2023).

With respect to the cholesterol-related effects observed in animals studies, i.e., fatty liver and hypercholesterolemia, VKM concluded that there were no human data confirming these findings. In one study increasing doses of NAC (up to 3600 mg per day for 4 weeks) resulted in increasing levels of high-density lipoprotein (HDL) while the concentration of other lipoproteins and lipids remain unaffected (Francheschini et al., 1993).
Two additional studies investigating the relationship between cysteine and lipid profile or body composition were identified (Costa et al., 2017; Elkafrawy et al., 2021).
Costa et al. (2017) studied the effect of high baseline serum homocysteine and cysteine levels on the lipid profile of children and adolescents (7 – 15 years) over a 12-month period in a prospective

cohort study. Study participants with high homocysteine or cysteine levels at baseline (≥5th quintile) showed a statistically significantly decrease in mean serum HDL-cholesterol and increase in mean triglyceride levels in follow-up period. When both homocysteine and cysteine serum levels were high at baseline, these changes were greater. This was irrespective of age, sex, socioeconomic status, diet or anthropometric status. The authors state that high homocysteine and cysteine levels may represent risk factors for chronic non-communicable diseases, like dyslipidemias.
Elkafrawy et al. (2021) studied the plasma sulfur amino acid and thiol profile in relation to body composition in 35 healthy, non-smoking young adults (mean age ± SEM males: 29 ± 1.6 years, females: 32 ± 2.4 years). They found that the fasting plasma concentrations of the free disulfide fractions of cysteine were strongly and independently associated with fat mass. In addition, exposure of human adipose-derived stem cells in vitro to increasing physiological concentrations of cystine dose-dependently enhanced adipogenesis and lipid accumulation. The authors concluded that increased cystine concentrations are positively associated with increased fat mass in humans and human adipogenic differentiation in vitro. Taken together, these studies suggest that high plasma levels of cysteine are correlated with increased body fat. It remains however unclear whether there is a causal relationship.

Natural medicines (2022) indicates that NAC is generally well-tolerated when used in typical doses (600-1200 mg per day), and that adverse effects occur when NAC is used in single doses of more than 9 grams or daily doses of more than 30 grams. The most common side effects that are mentioned include diarrhea, dry mouth, dyspepsia, heartburn, loss of appetite, nausea and vomiting (Natural medicines, 2022).

The Summary of Product Characteristics (SmPC) of acetylcysteine mentions the following side effects: allergic reaction (bronchospasms, dyspnoea, pruritus, urticaria, skin rash, angioedema and tachycardia), headache, tinnitus, abdominal pain, nausea, diarrhoea, vomiting, stomatitis, fever, decreased blood pressure (uncommon; ≥1/1000 - ≥1/100), dyspepsia (rare; ≥1/10000 - ≥1/1000) and anaphylactic shock and bleeding (very rare; <1/10000). In addition, it is mentioned that in several studies a decrease in platelet aggregation is observed, however the clinical relevance has yet to be established (MEB, 2023).

Table 4 Overview of oral human studies (published 2015-2021) investigating adverse health effects (AEs) of NAC.

Studies in adults

Reference	Participant characteristics, age groups	Number in treatment group NAC	Control	Doses	Main endpoints	Duration	Adverse effects
Sepehrmanesh et al. (2018)	Patients (18 to 65 years) with chronic schizophrenia	40	39	1200 mg per day (2x 600 mg)	To assess the clinical effect of NAC as an add-on to maintenance medication for treatment of chronic schizophrenia	12 weeks (double-blind RCT)	No effects on safety parameters (physical examination, electrocardiogram (ECG)), nor difference in frequency of reported AEs
Berk et al. (2019)	Patients with bipolar disorder and current depressive symptoms	59	61	2000 mg per day (2x 1000 mg)	To assess the efficacy of NAC as treatment in bipolar depression	16 weeks	The number of reported gastrointestinal issues (heartburn/reflex/indigestion) was statistically significantly higher in the NAC than in the placebo group
Coles et al. (2018)	Patients with Parkinson disease (n=5), healthy subjects (n=3)	8	-	6000 mg per day (2x 3000 mg)	To characterize NAC pharmacokinetics and to evaluate its effect on blood and brain glutathione following repeated oral dosing	4 weeks	AEs were noted in 3 of the 5 individuals with Parkinson disease and 1 of 3 healthy controls, and included mild indigestion, increased drooling, mild to moderate increases in tremor and in one case freezing of gait. All AEs were dissolved 2 weeks after NAC discontinuation.

Reference	Participant characteristics, age groups	Number in treatment group NAC	Control	Doses	Main endpoints	Duration	Adverse effects
Krysko et al. (2021)	Individuals with progressive multiple sclerosis with modified Fatigue Impact Scale (MFIS) > t38 (18-75 years)	10	5	1250 mg per day (2 x 625 mg)	To assess the feasibility, tolerability, and safety of NAC for fatigue in progressive MS	4 weeks	Serum creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) within normal limits for all patients. No statistically significant difference in the number of AEs between NAC and placebo group. No serious AEs reported. Two AEs in one patient attributed to NAC; abdominal pain and constipation.
Machado et al. (2020)	Tobacco use disorder patients (18-65 years)	17	17	1800 mg per day	To examine the efficacy and safety of NAC as an adjunctive treatment for smoking cessation.	12 weeks	No differences were found in either group regarding adverse events during the treatment period.
Rani, Aggarwal & Vohra (2020)	Patients (≥18 years old) fulfilling the NCEP-ATP III diagnostic criteria for metabolic syndrome	35	35 (compared with their own baseline)	1200 mg per day (2 x 600 mg), along with their ongoing therapeutic regimen	To evaluate the effect of NAC on the metabolic profile in metabolic syndrome	6 weeks	No serious AEs reported. Most common effects reported with NAC: headache (n=2), nausea/vomiting (n=1)

Reference	Participant characteristics, age groups	Number in treatment group NAC	Control	Doses	Main endpoints	Duration	Adverse effects
Skov et al. (2015)	Patients (25-61 years) with cystic fibrosis (CF) and chronic *Pseudomonas aeruginosa* lung infection	11	10	2400 mg per day (2x 1200 mg)	To investigate the effect of NAC on oxidative stress markers in urine and plasma antioxidant levels	30 days	One patient stopped treatment with NAC due to stomach pain. No other adverse events were observed.
Johnson et al. (2016)	COPD patients (41-85 years) with chronic cough and sputum production	23	22	3600 mg per day (2x 1800 mg)	To investigate whether high-dose NAC would improve respiratory health status	8 weeks	Most AEs unrelated to study drug. More nausea/diarrhoea reported in NAC group (5) compared to placebo (1). No statistically significant treatment-related differences in blood counts, electrolytes, creatinine, ALT, prothrombin time.
Monti et al. (2019)	Parkinson disease patients (40-80 years)	28	14	1200 mg per day (2x 600 mg) or an IV infusion once a week	To investigate whether combination of oral and IV NAC results in improvements in dopamine transporter binding and symptoms	90 days	No AEs throughout study period
Monti et al. (2020)	Multiple sclerosis patients (21-80 years)	24	24 (compared with their	1000 mg per day (2x 500 mg) or	To explore effects on cerebral glucose	~60 days	There were no severe AEs reported.

Reference	Participant characteristics, age groups	Number in treatment group		Doses	Main endpoints	Duration	Adverse effects
		NAC	Control				
			own baseline)	an IV infusion once a week	metabolism and symptoms		
Breier et al. (2018)	Chronic schizophrenia patients (16-35 years)	30	30	3600 mg per day	To assess therapeutic effects on total, negative, and positive symptoms, cognitive impairment and functioning in schizophrenia spectrum disorders	52 weeks	No statistically significant difference in the number of AEs between NAC and placebo group. Two subjects from each group reported an serious AE (worsening psychotic symptoms) and one subject from the NAC group reported asthma exacerbation. These were deemed to be unrelated to NAC.
Christensen & Bangsbo (2019)	Cyclists (28±7 years)	11	11	1500 mg	To evaluate effect in well-trained cyclists on intense endurance exercise performance and physiologic exercise response	Single dose, 60 min for test, 1 day	There were no AEs reported.
Ellegaard et al. (2019)	Patients with bipolar depression (18-64 years)	40	40	3000 mg per day	To investigate reducing symptoms of depression in	20 weeks	There were no statistically significant differences in AEs between NAC and placebo group. Most common AEs

Reference	Participant characteristics, age groups	Number in treatment group NAC	Control	Doses	Main endpoints	Duration	Adverse effects
					subjects with bipolar depression		included headache, diarrhoea, nausea, dizziness. Serious AEs included. anaemia, influenza, pneumonia, alcohol intoxication, increased suicidal ideation, psychiatric hospital admission
Grant, Chamberlain & Redden (2016)	Patients with skin-picking disorder (18-65 years)	35	31	1200 mg per day (first 3 weeks), 2400 mg per day (second 3 weeks), 3000 mg per day (remaining 6 weeks)	To investigate efficacy for reducing compulsive picking behaviour	12 weeks	There were no statistically significant differences in AEs between NAC and placebo group. Reported AEs were mild and included nausea, dry mouth, constipation, dizziness.

Studies in children

Reference	Participant characteristics, age groups	Number in treatment group		Doses	Main endpoints	Duration	Adverse effects
Bloch et al. (2013)	Trichotillomania patients (8-17 years)	20	19	2400 mg per day (2x 1200 mg)	To examine efficacy for treatment of pediatric trichotillomania	12 weeks	There were no statistically significant differences in AEs between NAC and placebo group. One case of full body rash in NAC group.
Li et al. (2020)	Patients with obsessive-compulsory	5	6	2700 mg per day (3x 900 mg)	To investigate effect for pediatric	12 weeks	There were no statistically significant differences in AEs between NAC and placebo

Reference	Participant characteristics, age groups	Number in treatment group	Doses	Main endpoints	Duration	Adverse effects
	disorder (8-17 years)			obsessive-compulsive disorder		group. One subject in NAC group reported skin rash, this was not thought to be medication related.

5.2.8 Interactions

In general, it is advised to not simultaneously ingest NAC with other human medicines (Farmacotherapeutisch Kompas, 2022a).

NAC should not be concomitantly used with medication that reduces irritation of the throat, because a diminished reflex to cough may lead to accumulation of bronchial secretion (Farmacotherapeutisch Kompas, 2022a).

It is suggested that concomitant use of NAC and activated charcoal reduces the effect of each other (Natural Medicines, 2022; Farmacotherapeutisch Kompas, 2022a).

Acetylcysteine may lower the plasma level of carbamazepine and thereby increase the risk of seizures (Farmacotherapeutisch Kompas, 2022a).

In vitro it has been noted that blending acetylcysteine with antibiotics lead to an inactivation of the antibiotics. As a precautionary principle it is therefore advised to not take antibiotics at the same time but 2 hours before or after the intake of NAC (Farmacotherapeutisch Kompas, 2022a).

Acetylcysteine may increase the vasodilating effect of nitroglycerine (and isosorbide) resulting in hypotension and headaches, and it is therefore strongly advised not to use concomitantly (Farmacotherapeutisch Kompas, 2022a; Natural Medicines, 2022; VKM, 2015). Also, it may theoretically increase the risk of hypotension when concomitantly taken with antihypertensive drugs or herbs and supplements with hypotensive effects (Natural Medicines, 2022).

In addition, NAC might theoretically increase the risk of bleeding when concomitantly taken with anticoagulant or antiplatelet drugs, because a decreased prothrombin time, decreased platelet aggregation, prolonged coagulation time and increased blood loss in surgical patients have been suggested in clinical research. The same applies to herbs and supplements that have an anticoagulant or antiplatelet effect (Natural Medicines, 2022).

VKM (2015) also mentioned that NAC may strengthen the effects of immune suppressants, such as azathioprine, cyclophosphamide, or prednisone.

5.3 Derivation of toxicological reference value

VKM (2015) used a dose of 900 mg per day for L-cysteine and L-cystine, corresponding to 13 mg/kg bw per day for a 70-kg adult, as value for comparison in the risk characterization. This was based on the absence of adverse effects at a daily dose of 1200 mg NAC in human studies in various population groups (VKM, 2015). Assuming similar tolerance for children (10 – <14 years) and adolescents (>14 – 18 years), VKM also used the value of 13 mg/kg bw per day for the risk characterization for these age groups.

The information in human studies published since the VKM opinion does not give cause to alter the conclusion that 13 mg/kg bw per day L-cysteine or L-cystine is a safe dose for adults, adolescents and children (10 – <14 years).
It is noted that this is higher than the therapeutic dose for NAC of 600 mg per day, corresponding to 450 mg L-cysteine[14] or 6.4 mg/kg bw for a 70-kg adult. However, the therapeutic effect of NAC being a mucolytic agent is not considered adverse. Hence, in the current assessment the value of VKM will be used as value for comparison for the risk assessment.

VKM did not include children under 10 years of age in their risk assessment. For children of 2 – 10 years, the therapeutic dose for NAC is higher than the value of VKM of 13 mg/kg bw per day. Therefore, it is considered justified to use the value of VKM for children of these ages as well.

For children younger than 2 years, the use of NAC is contraindicated (see also section 6.3). Therefore, for this age group no safe dose or reference value can be derived. Based on default body weights for children (te Biesebeek et al., 2014), a dose of 13 mg/kg bw per day L-cysteine or L-cystine equals 190 mg/day for a 2-6 year old child, 320 mg/day for a 6-11 year old child and 580 mg/day for a 11-18 year old child (see Table 5).

Table 5 Toxicological reference values for L-cysteine and L-cystine and NAC for different age groups

Age group (years)	Average body weight (kg)	Reference value L-cysteine and L-cystine (mg/kg bw per day)	Reference value L-cysteine and L-cystine (mg/day)	Reference value NAC (mg/day)
>18	70[a]	13	900	1200
11 – 18	44.8[b]	13	580	770
6 – 11	24.3[b]	13	320	430
2 – 6	14.3[b]	13	190	250
0 – 2 years	NA[c]	NA[c]	NA[c]	NA[c]

[a] Default body weight for adults used by VKM (2015).
[b] Default values for body weight of children aged, respectively, 11-16, 6-11 and 2-6 year reported by Te Biesebeek et al. (2014).
[c] Not applicable. For this age group, the use of NAC as a therapeutic agent is contraindicated and therefore no reference value could be derived.

[14] Or L-cystine

6 Risk assessment

6.1 Risk assessment

The daily exposure to NAC or L-cysteine from the use of food supplements varies depending on the food supplement used. The estimated exposure to NAC ranged between 500 to 2000 mg per day corresponding to 7.1-29 mg/kg bw per day for an adult of 70 kg when food supplements containing NAC are used. This yields an exposure to L-cysteine of approximately 375 to 1500 mg[15], corresponding to 5.4-21 mg/kg bw per day for an adult of 70 kg. When food supplements containing L-cysteine are used the exposure to L-cysteine amounts to 500 to 1500 mg per day corresponding to 7.1 – 21 mg/kg bw per day. For adults the use of part of the food supplements containing NAC and/or L-cysteine leads to an exposure above the reference value of 13 mg/kg bw per day. Accordingly, in these cases the development of gastrointestinal complaints or other side effects as mentioned for medicinal products cannot be ruled out.

The same holds for children of 2 years and older. For this age group, exceedance f the 13 mg/kg bw per day will occur for more food supplements since, due to their lower body weights, the relative exposure per kg body weight will be higher.

For children under 2 years of age the use of NAC is contraindicated due to their inability to cough up mucus. Therefore, the use of food supplements containing NAC, L-cysteine or L-cystine for this age group is considered not safe.

Besides exposure resulting from food supplement use, relatively low exposure to L-cysteine occurs via food because it is a component of food proteins and it is authorized for use as a food additive and flavouring agent.

In addition, when NAC is also used as a mucolytic agent (medicine) an additional exposure to L-cysteine of 450 mg (for children >7 years and adults) or 300 mg (children aged 2-7 years)may take place (see section 4.2). Therefore, when food supplements and medicines containing NAC and/or L-cysteine or L-cystine are combined there is a higher risk for the development of gastrointestinal complaints or other side effects.

6.2 Interactions

Concomitant use of NAC with other medication that reduces irritation of the throat is advised against because a diminished cough reflex could lead to accumulation of bronchial secretion (Farmacotherapeutisch Kompas, 2022a). Other combinations that are mentioned to result in an interaction are concomitant use of NAC with activated charcoal, carbamazepine, antibiotics, and nitroglycerine.

[15] Calculated based on molecular weights (see section 3.1)

6.3 Sensitive/vulnerable groups

NAC is contraindicated in children under 2 years of age, because of their inability to cough up mucus (Farmacotherapeutisch Kompas, 2022a). In addition, caution is warranted when suffering from asthma or COPD due to the chance on bronchospasms. In the case bronchospasms or changes to the skin or mucosae occur, it is advised to stop using NAC (Farmacotherapeutisch Kompas, 2022a). Caution is also advised when suffering from gastric ulcer due to the possible irritation of the mucosa of the gastrointestinal tract (Farmacotherapeutisch Kompas, 2022a). Further, since NAC may have an effect on histamine metabolism, caution is warranted when using NAC for a longer period of time when suffering from histamine intolerance (Farmacotherapeutisch Kompas, 2022a).

In individuals with severe liver injury or end stage renal disease, the kinetics of NAC may be altered due to lower clearance resulting in higher systemic exposure (Tenório et al., 2021) and thereby they may be more prone to developing side effects.

Individuals who are diagnosed with the hereditary disease cystinuria, in which kidney stones are formed from circulating cystine, should consult a physician when they want to use NAC (VKM, 2015).

6.4 Uncertainties

6.4.1 Exposure

The exposure to NAC, L-cysteine or L-cystine is estimated based on information provided on the available information online and/or the leaflet of the product and takes into account the recommended use. As there is no pre-market quality control for food supplements, the precise composition may differ between batches. This leads to an uncertainty in the exposure estimation. Further, use exceeding the recommended dose will lead to higher exposure to NAC, L-cysteine and L-cystine.

6.4.2 Toxicity

As mentioned by VKM (2015) in their report, the human data is mainly from RCTs that are designed to study the beneficial effects of NAC. Adverse effects are most of the time self-reported effects. It is therefore unknown if changes in body parameters like for example changes in blood lipids, occur following NAC treatment. In animal studies high doses of NAC have been reported to result in fatty liver and hypercholesteremia. This is so far not reported in humans.
VKM also mentioned the lack of studies investigating the effect of high doses for longer periods (>6-12 months). In addition, data for children and adolescents are relatively scarce.

7 Conclusions and recommendations

In 2015, VKM determined a reference value for L-cysteine of 13 mg/kg bw per day, that is considered to not lead to appreciable health risks. The review of recent human studies does not warrant an adjustment of this value. This reference value equals doses of 900 mg/day L-cysteine or L-cystine corresponding to 1200 mg/day NAC for adults. For children aged 11-18 years this amounts to 580 mg/day L-cysteine or L-cystine and 770 mg/day NAC. For children aged 6-11 years, this equals 320 mg/day L-cysteine or L-cystine and 430 mg/day NAC, and for children aged 2-6 years 190 mg/day L-cysteine or L-cystine and 250 mg/day NAC. Food supplements with a recommended daily dose below these reference values are therefore considered to not lead to health risks.

For children under 2 years of age the use of NAC as a medicine is contraindicated and therefore, the use of food supplements containing NAC, L-cysteine or L-cystine is considered not safe for this age group.

When comparing the estimated exposure based on recommended use to the reference value, part of the food supplements available on the Dutch market leads to an exposure to L-cysteine that is higher than 13 mg/kg bw per day. This means that in these cases the development of gastrointestinal complaints or other side effects as mentioned for medicinal products cannot be ruled out.

It is not recommended to concomitantly use medicines containing NAC and food supplements containing NAC, L-cysteine and/or L-cystine, since this will lead to exceeding the reference value of 13 mg/kg bw per day.

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Annex I Search strategy

#1	"cystein*"[Title] OR "l cystein*"[Title] OR "cystin*"[Title] OR "acetylcystein*"[Title] OR "n acetylcystein*"[Title] OR "n acetyl cystein*"[Title]	32,333
#2	"risk*"[Text Word] OR "safety"[Text Word] OR "adverse"[Text Word] OR "side effect*"[Text Word] OR "hazard*"[Text Word] OR "harm*"[Text Word] OR "negative"[Text Word] OR "contraindicat*"[Text Word] OR "contra indicat*"[Text Word] OR "interact*"[Text Word] OR "toxicity"[Text Word] OR "toxic"[Text Word]	9,485,474
#3	#1 AND #2	8,933
#4	"homocysteine*"[All Fields] NOT ("homocystein*"[All Fields] AND ("cystein*"[All Fields] OR "cystin*"[All Fields] OR "acetylcystein*"[All Fields] OR ("n"[All Fields] AND "acetylcystein*"[All Fields]) OR ("n"[All Fields] AND ("acetyl"[All Fields] OR "acetylate"[All Fields] OR "acetylated"[All Fields] OR "acetylates"[All Fields] OR "acetylating"[All Fields] OR "acetylation"[MeSH Terms] OR "acetylation"[All Fields] OR "acetylations"[All Fields] OR "acetyls"[All Fields]) AND "cystein*"[All Fields]))	25,040
#5	#3 NOT #4	8,933
#6	((conference abstract*[Publication Type]) OR (letter*[Publication Type])) OR (editorial*[Publication Type])	1,902,298
#7	#5 NOT #6	8,760
#8	Limit 7 to (Danish or English or Norwegian or Swedish or Dutch or German)	8,570
#9	Limit 8 to (2015-2023)	3,193

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