



VKM Report 2016: 58

Risk assessment of "other substances" – glycine

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

Report from the Norwegian Scientific Committee for Food Safety (VKM) 2016: 58
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12.12.2016

ISBN: 978-82-8259-248-2
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Cover photo: iStock Photo

Suggested citation: VKM. (2016). Risk assessment of "other substances" – glycine. Opinion of the Panel on Nutrition, dietetic products, Novel Food and Allergy of the Norwegian Scientific Committee for Food Safety, ISBN: 978-82-8259-248-2, Oslo, Norway

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Acknowledgment

The Panel on Nutrition, Dietetic Products, Novel Food and Allergy has answered the request from the Norwegian Food Safety Authority. Project leader from the VKM secretariat has been Bente Mangschou. Grethe S. Tell is acknowledged for her valuable work on this opinion. Jan Alexander (the Scientific Steering Committee), Åshild Krogdahl (the Scientific Steering Committee) and Helle Margrete Meltzer (former member of the Panel on Nutrition, Dietetic Products, Novel Food and Allergy) constituted a reference group and are acknowledged for their valuable comments and suggestions on this opinion.

Competence of VKM experts

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

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Summary

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

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

The present report is a risk assessment of specified doses of glycine in food supplements, and it is based on previous risk assessments and articles retrieved from two literature searches.

Glycine is a non-essential amino acid which is synthesised from 3-phosphoglycerate via serine, or derived from threonine, choline and hydroxyproline via inter-organ metabolism involving primarily the liver and kidneys. Endogenous synthesis is estimated to be in the magnitude of 8 g per day in adults. Glycine is a constituent of all proteins in the human body. It also functions as a neurotransmitter, and can play both stimulatory and depressant roles in the brain. Data on dietary intake of glycine in Norway are not available. Based on NHANES III (1988-1994), the overall mean intake of glycine from food and food supplements in the United States was 3.2 g per day. Thus, the combined dietary intake and endogenous synthesis is more than 11 g per day. Because glycine is not considered an essential amino acid, a dietary requirement in healthy humans has not been established. Foods rich in glycine are generally protein rich foods such as meat, fish, dairy products and legumes.

According to information from NFSA, glycine is an ingredient in food supplements sold in Norway. NSFA has requested a risk assessment of 20, 50, 100, 300, 500 and 650 mg/day of glycine from food supplements.

There is a lack of relevant supplementation studies with glycine in humans designed to address adverse effects and/or dose-response relationship, and none of the previous reports reviewed concluded with a no observed adverse effect level (NOAEL). For the current risk assessment, two literature searches were conducted, one for human studies and one for animal studies. No human studies were found that can be used for suggesting a "value for comparison", and there are no scientific data in the published literature suitable for assessing the specific doses in the terms of reference.

The value for comparison used in this risk characterisation is 20 mg/kg per day. This value is derived from a study in rats in which the NOAEL was estimated at 2000 mg/kg per day. Using an uncertainty factor of 100, this corresponds to 20 mg/kg per day or 1.4 g per day for a person weighing 70 kg. This is more than twice as high as the highest dose for consideration in the present risk assessment, and it is far below the combined dietary intake and endogenous synthesis estimated at more than 11 g per day.

No particular vulnerable groups for glycine supplements have been identified.

VKM concludes that:

- In adults (≥ 18 years), the specified doses 20, 50, 100, 300, 500 and 650 mg/day of glycine from food supplements are unlikely to cause adverse health effects.
- In adolescents (14 to < 18 years), the specified doses 20, 50, 100, 300, 500 and 650 mg/day of glycine from food supplements are unlikely to cause adverse health effects.
- In children (10 to < 14 years), the specified doses 20, 50, 100, 300, 500 and 650 mg/day of glycine from food supplements are unlikely to cause adverse health effects.

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

Short summary

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

- In adults (≥ 18 years), the specified doses 20, 50, 100, 300, 500 and 650 mg/day of glycine from food supplements are unlikely to cause adverse health effects.
- In adolescents (14 to < 18 years), the specified doses 20, 50, 100, 300, 500 and 650 mg/day of glycine from food supplements are unlikely to cause adverse health effects.
- In children (10 to < 14 years), the specified doses 20, 50, 100, 300, 500 and 650 mg/day of glycine from food supplements are unlikely to cause adverse health effects.

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

Sammendrag på norsk

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

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

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

Glysin er en ikke-essensiell aminosyre som primært dannes fra 3-fosfoglycerat via serin, og den kan også dannes fra treonin, cholin og hydroksyprolin, primært i lever og nyrer. Endogen syntese er estimert til å være i størrelsesorden 8 g per dag hos voksne. Glysin er del av alle kroppens proteiner. Den er en neurotransmitter, og kan virke både som stimulator og hemmer i hjernen. Det finnes ikke norske tall for inntak av glysin i kosten. Ifølge data fra NHANES III (1988-1994) var gjennomsnittlig inntak av glysin fra mat og tilskudd i USA 3,2 g/dag. Totalt vil dermed inntak av glysin fra kosten samt bidrag fra endogen syntese utgjøre mer enn 11 g glysin per dag. Behov fra kosten er ikke fastsatt siden glysin ikke betraktes som en essensiell aminosyre. Matvarer med et høyt innhold av glysin er proteinrike matvarer som kjøtt, meieriprodukter, bønner og fisk.

Ifølge informasjon fra Mattilsynet er glysin en ingrediens i kosttilskudd som selges i Norge. Oppdraget fra Mattilsynet var å risikovurdere følgende doser av glysin i kosttilskudd: 20, 50, 100, 300, 500 og 650 mg/dag.

Ingen av de tidligere rapportene for glysin konkluderte med en NOAEL (no observed adverse effect level). For denne risikovurderingen har vi gjort to litteratursøk, ett for studier på mennesker og ett for dyrestudier. Ingen relevante humanstudier ble funnet som kan brukes til å vurdere risiko av glysin i henhold til doser. Kun en dyrestudie, på rotter, fant en NOAEL på 2000 mg/kg per dag. Ved å bruke en usikkerhetsfaktor på 100, tilsvarer dette 20 mg/kg per dag for mennesker, tilsvarende 1,4 g per dag for et menneske på 70 kg. Dette er over dobbelt så høyt som den høyeste dosen som skal vurderes i denne risikovurderingen, og mye lavere enn kombinasjonen av inntak fra kosten og endogen syntese, til sammen estimert som mer enn 11 g per dag.

Vitenskapskomiteen for mattrygghet (VKM) konkluderer med at:

- For voksne (≥ 18 år) er det usannsynlig at de spesifiserte dosene på 20, 50, 100, 300, 500 og 650 mg/dag glysin i kosttilskudd vil forårsake negative helseeffekter.
- For ungdom (14 til < 18 år) er det usannsynlig at de spesifiserte dosene på 20, 50, 100, 300, 500 og 650 mg/dag glysin i kosttilskudd vil forårsake negative helseeffekter.
- For barn (10 til < 14 år) er det usannsynlig at de spesifiserte dosene på 20, 50, 100, 300, 500 og 650 mg/dag glysin i kosttilskudd vil forårsake negative helseeffekter.

Barn under 10 år inngår ikke i dette oppdraget.

Kort sammendrag

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

- For voksne (≥ 18 år) er det usannsynlig at de spesifiserte dosene på 20, 50, 100, 300, 500 og 650 mg/dag glysin i kosttilskudd vil forårsake negative helseeffekter.
- For ungdom (14 til < 18 år) er det usannsynlig at de spesifiserte dosene på 20, 50, 100, 300, 500 og 650 mg/dag glysin i kosttilskudd vil forårsake negative helseeffekter.
- For barn (10 til < 14 år) er det usannsynlig at de spesifiserte dosene på 20, 50, 100, 300, 500 og 650 mg/dag glysin i kosttilskudd vil forårsake negative helseeffekter.

Abbreviations and glossary

Abbreviations

AESAN	- Spanish Agency for Food Safety and Nutrition
bw	- body weight
EFSA	- European Food Safety Authority
GCS	- glycine cleavage system
IOM	- Institute of Medicine, USA
LOAEL	- lowest observed adverse effect level
NFSA	- Norwegian Food Safety Authority [<i>Norw.</i> : Mattilsynet]
NMDAR	- N-methyl-D-aspartate receptor
NOAEL	- no observed adverse effect level
RCT	- randomised controlled trial
SAFTEE	- Systematic Assessment For Treatment Emergent Events
SEM	- standar error of the mean
SOPS	- Scale Of Psychosis-risk Symptoms
UL	- tolerable upper intake level
VKM	- Norwegian Scientific Committee for Food Safety [<i>Norw.</i> : Vitenskapskomiteen for Mattrygghet]
WHO	- World Health Organization

Glossary

"Other substances": a substance other than a vitamin or mineral that has a nutritional or physiological effect (European Regulation (EC) No. 1925/2006, Article 2; <http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R1925&from=en>).

"Negative health effect" and "adverse health effect" are broad terms. The World Helath Organization (WHO) has established the following definition of "adverse effect": a change in morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences (WHO, 1994).

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

Background as provided by the Norwegian Food Safety Authority

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

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

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

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

Terms of reference as provided by the Norwegian Food Safety Authority

The Norwegian Food Safety Authority (NFSA) requested the Norwegian Scientific Committee for Food Safety (VKM) to assess the safety of glycine in food supplements at the following doses: 20, 50, 100, 300, 500 and 650 mg/day.

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

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

1 Introduction

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

This risk assessment concerns the substance glycine per se, and no specific products.

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

According to information from the Norwegian Food Safety Authority (NFSA), glycine is an ingredient in food supplements sold in Norway. NFSA has requested a risk assessment of the intake of 20, 50, 100, 300, 500 and 650 mg glycine per day from food supplements. Glycine exposure from other sources than food supplements is not included in the risk assessment.

Glycine is a non-essential amino acid. It is synthesised from 3-phosphoglycerate via serine and may also be derived from threonine or other endogenous metabolites in the liver. In adults with an adequate protein intake, endogenous synthesis is sufficient to cover physiological needs. In adult men (mean (\pm SEM) age 21.5 ± 2.7 years) consuming daily 44 kcal of energy and 1.5 g protein per kg body weight, the rate of whole-body glycine synthesis has been estimated to be 116 mg/kg body weight per day, corresponding to 8.1 g/day in a 70 kg person (Yu et al, 1985). Further, the same study found a reduced endogenous production of glycine in response to decreased intakes of both nutritionally essential and non-essential amino acids.

Foods rich in glycine are generally protein rich foods such as meat, fish, dairy products and legumes. Based on distribution data from the 1988–1994 NHANES III, the mean daily intake for all life stage and gender groups of glycine from food and supplements is 3.2 g/day. Men 19 through 30 years of age had the highest intakes at the 50th percentile of 4.6 g/day and at the 99th percentile of 7.8 g/day (IOM, 2005).

2 Hazard identification and characterisation

2.1 Literature

This risk assessment is based on previous risk assessments of glycine, as well as scientific papers retrieved from a systematic search in literature published before 5 April 2016. The literature search aimed at retrieving human studies on adverse effects caused by glycine. A search for animal studies investigating possible adverse effects caused by glycine was performed on 2 August 2016.

2.1.1 Previous risk assessments

Risks related to glycine supplementation have previously been evaluated by the Institute of Medicine (IOM), USA in 2005 and VKM 2011.

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

IOM attempted to establish an upper tolerable intake level (UL) for amino acids in 2005, including glycine. Both animal and human studies were reviewed. The IOM report did not conclude regarding possible adverse effects of supplementation in humans, and stated that "the data on adverse effects of glycine intake from supplements were considered not sufficient for a dose–response assessment and derivation of a UL."

Regarding possible adverse effects in animals, the report stated: "Growth depression in rats and chicks has been reported after feeding diets containing as much as 10 percent glycine (Harper et al., 1970). Nitrosated glycine can be genotoxic in vitro (Gaspar et al., 1996). It is not, however, mutagenic using a modified Ames test (Hoorn, 1989)."

The IOM report concluded that none of the studies, human or animal, could be used to establish a no observed adverse effect level (NOAEL) or a lowest observed adverse effect level (LOAEL) (IOM, 2005).

VKM report on risk categorisation of amino acids, Norway, 2011

In 2011 VKM conducted a risk categorisation of about 30 amino acids and amino acid compounds according to potential health risks related to high intakes of the amino acids (VKM, 2011). This categorisation was based on a comprehensive MEDLINE literature search including both human and animal studies. No human studies focusing on potential negative health effects from oral supplementation with glycine were identified. No animal studies of glycine were found relevant for inclusion.

Because glycine has produced changes in biomarkers with known negative health effects, it was categorised by VKM as an amino acid with moderate risk. The rationale for this classification was not provided in the report. It was emphasised that the VKM report from 2011 has several limitations and can only be regarded as a preliminary report and not as a risk assessment of amino acids.

2.1.2 Literature search

2.1.2.1 Search strategy

Literature searches were performed in MEDLINE and EMBASE in order to retrieve publications on adverse effects caused by glycine. Both databases were searched to ensure comprehensive study retrieval. The literature search for human studies was conducted 5 April 2016. A literature search for animal studies was conducted on 2 August 2016. The strategies for the searches are outlined in Appendix 1.

2.1.2.2 Publication selection and data extraction

In the literature search for human studies 637 titles and abstracts were identified, the corresponding number for animal studies was 1095. In the primary screening, titles and abstracts of all unique publications retrieved were independently screened against the inclusion criteria.

Inclusion criteria:

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

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

The inclusion criteria checklist was developed by members of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics and the Panel on Nutrition, Dietetic Products, Novel Food and Allergy. Titles and abstracts that did not fulfil the inclusion criteria were excluded from further screening. In situations where it was unclear whether the publication was of relevance to the current risk assessment, it was retained for further screening. The primary screening for human studies was performed by the author, and the primary screening for animal studies was performed independently by two persons (member of the Panel and coordinator from the secretariat).

The publications that passed the primary screening were reviewed in full text against the same inclusion criteria by the author of this report.

The first screening of the literature search for human studies resulted in 4 full text articles, while the full-text review resulted in one relevant article. Additionally, nine human studies from manual search were identified and reviewed. Of these, two human studies were found potentially relevant and included in this review. The screening for animal studies resulted in two full text articles of which only one was relevant and included in this report. (see Figure 2.1.2.2-1).

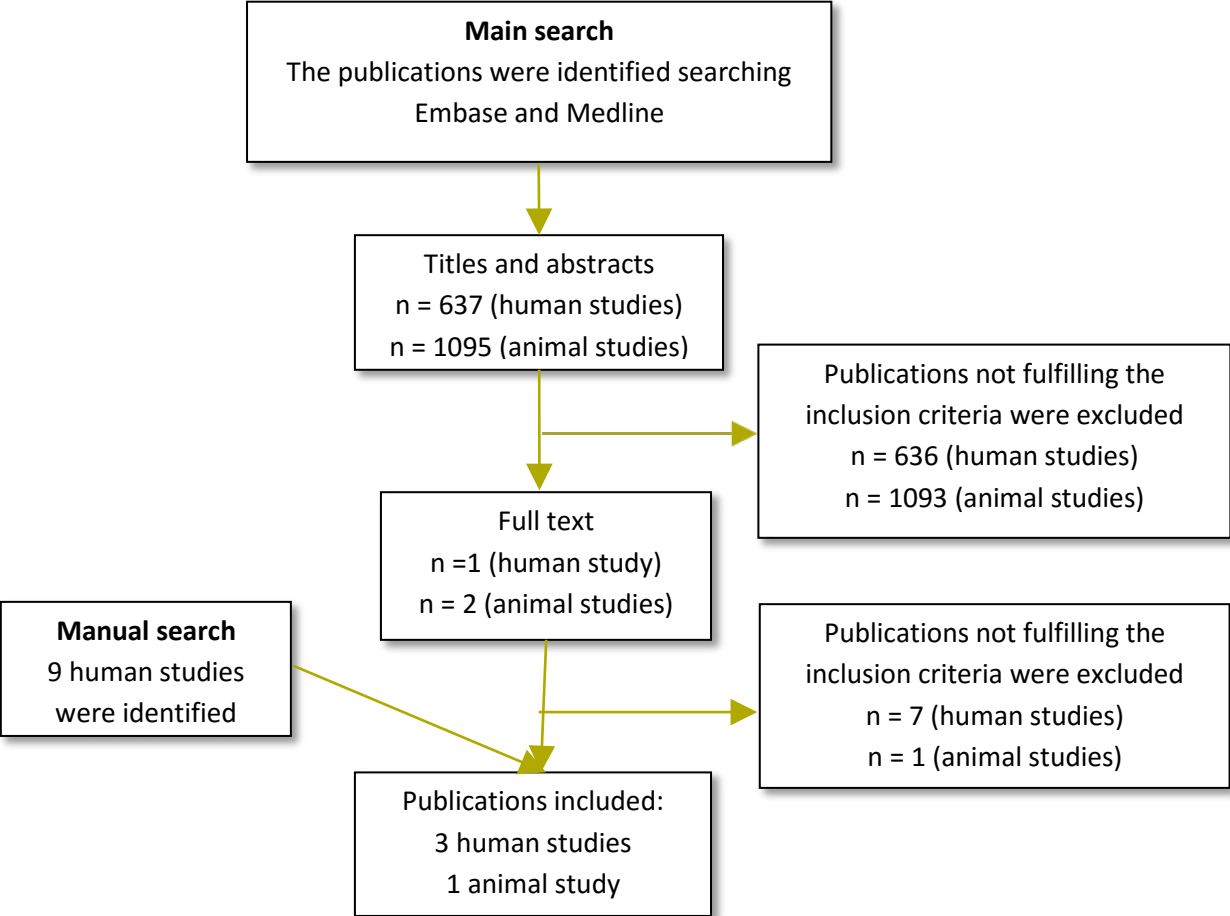


Figure 2.1.2.2-1: Flowchart for publication selection for human studies of glycine.

2.2 General information

2.2.1 Chemistry

Glycine, also named aminoethanoic acid, aminoacetic acid and glycocoll, is non-essential and the smallest of the amino acids. Glycine was first isolated from gelatin in 1820, and is manufactured industrially by treating chloroacetic acid with ammonia. The molecular formula is $C_2H_5NO_2$. The CAS number for glycine is 56-40-6. The structural formula is shown in figure 2.2.1-1.

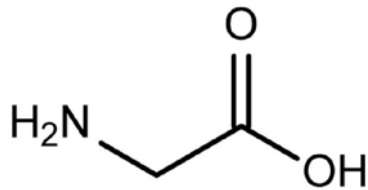


Figure 2.2.1-1: Structural formula of glycine.

2.2.2 Occurrence

Food sources generally rich in glycine are protein-rich foods such as meat, fish, dairy products and legumes. Glycine is also found in high amounts in gelatin, a substance made from collagen. Dietary sources of gelatin include gelatin desserts and gummy candy. Because of its sweet properties, it is also an additive (E 640 – acceptable daily intake (ADI not specified)) in various food products, such as yogurt, cream cheese, margarine and ice cream.

In a 70 kg male with moderate lipid stores, body protein level is about 15% (i.e. 10.5 kg protein) of which 10% is estimated to be glycine (based on data from growing pigs (Bikker et al., 1994), i.e. 1000-1100 g glycine. According to information from NFSA, glycine is an ingredient in food supplements sold in Norway, in the doses 20, 50, 100, 300, 500 and 650 mg/day.

2.3 Absorption, distribution, metabolism and excretion

2.3.1 In humans

Glycine is produced in the liver and kidneys from 3-phosphoglycerate via serine, and may also be derived from threonine, choline and hydroxyproline. It is substrate for the biosynthesis of nucleic acids as well as of bile acids, porphyrins, creatine phosphate, and other amino acids and proteins. In addition glycine is a major source of methyl group donation in the one carbon metabolism that involves the folate and methionine cycle and is hence involved in the biosynthesis of lipids, nucleotides and proteins as well as the maintenance of redox status (Anderson et al., 2012). Glycine is considered a principal

building block in proteins, such as its periodically repeated role in the formation of the collagen helix in conjunction with hydroxyproline. On a molar basis, glycine is the second most common amino acid found in proteins and enzymes. Glycine has the ability to inhibit neurotransmitter signals in the peripheral and central nervous system. Conversely, this amino acid also acts as an excitatory co-agonist via the N-methyl-D-aspartate subtype of glutamate receptors (NMDARs) (Bannai et al., 2012). Glycine may have anti-inflammatory effects during ischemia, injury, and transplantation (Bannai et al., 2012). Finally, recent genetic and functional evidence suggests that hyperactivation of the glycine-dependent one carbon pathway is a possible driver of oncogenesis and establishes links to cellular epigenetic status. Based on the number of clinically available agents that target one carbon metabolism, it has been suggested that targeting glycine-dependent one-carbon metabolism can be applied in precision cancer medicine (Locasale, 2013).

The half-life of glycine and its elimination from the body varies based on dose and metabolic need. In one study, the half-life was between 0.5 and 4.0 hours (Hahn, 1993). As described by Wang et al. (2013), glycine degradation occurs through three pathways: the glycine cleavage system (GCS), serine hydroxymethyl-transferase, and conversion to glyoxylate by peroxisomal D-amino acid oxidase. Among these pathways, GCS is the major enzyme system to initiate glycine degradation to form ammonia and CO₂ in animals. In addition, glycine is utilised for the biosynthesis of glutathione, heme, creatine, nucleic acids, and uric acid. Furthermore, glycine is a significant component of bile acids secreted into the lumen of the small intestine that is necessary for the digestion of dietary fat and the absorption of long-chain fatty acids. Glycine plays an important role in metabolic regulation, anti-oxidative reactions, and neurological function.

2.4 Toxicological data/Adverse effects

2.4.1 Human studies

The literature search identified one relevant study evaluating the effect of glycine treatment. Two additional potentially relevant studies were identified during manual search.

Because glycine may inhibit neurotransmitter signals in the central nervous system, it has been studied among patients at risk of psychosis and as add-on treatment for schizophrenia. Only one study, of patients at risk of psychosis, was considered relevant.

Another area where the effect of glycine supplementation has been studied is in relation to sleep quality. Because glycine plays a role as an allosteric modulator for the N-methyl-D-aspartate (NMDA) receptor, thereby causing hypothermia and vasodilation, it has been postulated to improve sleep quality (Bannai et al., 2012).

No studies were identified that evaluated possible dose-response effects of glycine.

Table 2.4.1-1: An overview of human studies investigating glycine and adverse health effects.

Reference	Study design/participant characteristics	Country	Number in treatment group		Dose	Main endpoint	Length of follow-up or duration of the study	Adverse effect
			Intervention	Control				
Woods et al. (2013)	Patients with the Risk Syndrome for Psychosis Open-label Pilot study	USA	10	0	Increasing, up to 0.8 g/kg/d as bulk powder	Psychosis symptoms	8 weeks on treatment, plus 16 weeks follow-up	"Systematic Assessment For Treatment Emergent Events", vital signs, weight, and laboratory evaluation 'No safety concerns were identified'
Same reference as above	RCT Pilot study	USA	4	4	Increasing, up to 0.8 g/kg/d as 'sprinkles'	Psychosis symptoms. Neurocognitive battery	12 weeks, followed by open-label Gly for 12 weeks	"Systematic Assessment For Treatment Emergent Events", vital signs, weight, and laboratory evaluation 'No safety concerns were identified'. How information on this was obtained is not specified.
Inagawa et al. (2006)	RCT cross-over of 15 female patients with sleeping problems	Japan	15	15	3 g/d	Subjectively reported sleep quality	4 days, with 3 days wash-out in between	No serious adverse events were observed. No mention of how this information was obtained.
Yamadera et al. (2007)	Randomised single-blind crossover trial of 11 volunteers with sleep problems	Japan	11	11	3 g/d	Subjective and objective measures of sleep	2 nights, with 1 week wash-out in between	No serious adverse effects were observed, assessed through a clinical interview, not further defined.

An additional randomised controlled trial (RCT) by Khan et al., from 2006 was identified in the literature search, but was not included because the reported doses appeared implausibly high (298 g 2 times/day), and VKM has not succeeded in obtaining a response from the authors concerning whether the dose unit was erroneously reported.

The literature search did not identify any relevant studies in children.

2.4.1.1 Randomised controlled trials

Glycine treatment of the risk syndrome for psychosis: Report of two pilot studies. Woods et al., 2013

The purpose of this study was to determine which symptoms or domains of cognition show the greatest response to glycine in risk syndrome patients (patients at high risk of developing psychosis) (Woods et al., 2013). The authors conducted two short-term pilot studies of glycine used without adjunctive antipsychotic medication. In the first trial, ten treatment-seeking outpatients 14-35 years old who met diagnostic criteria for a possible risk syndrome (Criteria of Psychosis-risk Syndromes (COPS)) received open-label glycine at doses titrated to 0.8 g/kg/day for 8 weeks, followed by discontinuation and 16 weeks of evaluation for durability of effects. In the second trial, 8 similar subjects were randomised to double-blind glycine vs. placebo for 12 weeks, followed by open-label glycine for another 12 weeks. Patients were evaluated every 1–2 weeks with the Scale Of Psychosis-risk Symptoms (SOPS) and before and after treatment with a neurocognitive battery. In the first study, glycine was dispensed as powder in prescription vials with instructions to dissolve the full contents of one vial in 8 ounces of fluid and take by mouth twice daily with meals. In the second study, glycine was administered as 'sprinkles' to be spooned over pudding or applesauce and swallowed with minimal chewing. In both studies glycine dosing was fixed at an initial dose of 0.2 g/kg every night at bedtime for 3 days, then 0.2 g/kg twice daily for 4 days, then 0.2 g/kg in the morning and 0.4 g/kg in the evening for 4 days, and finally 0.4 g/kg twice daily.

Safety was assessed by analysing treatment-emergent adverse events (Systematic Assessment For Treatment Emergent Events, SAFTEE, specific inquiry method), vital signs, weight, and laboratory evaluations. Treatment-emergent adverse events were defined as those occurring at the moderate level or higher at any time point and representing an increase over baseline. Several "adverse events", not defined, emerged during treatment in the open-label pilot; however, none were identified when glycine was given in a double-blind fashion, and some occurred among subjects on placebo.

The authors concluded that "No safety concerns were identified".

Subjective effects of glycine ingestion before bedtime on sleep quality. Inagawa K, et al., 2006

The purpose of this study was to examine the effects of glycine on sleep quality in a randomised double-blinded cross-over trial (Inagawa et al., 2006). Fifteen female volunteers with complaints about their sleep quality ingested either glycine (3 g) or placebo before bedtime, for 4 days, with 3 days wash-out in between. The women's subjective feeling in the following morning was evaluated with the St. Mary's Hospital Sleep Questionnaire and Space-Aeromedicine Fatigue Checklist. These results suggest that glycine produced a good subjective feeling after awakening from sleep. The authors reported that "No serious adverse events of the study protocol, including glycine and placebo intake, were observed during the study period". How this information was obtained was not described.

Glycine ingestion improves subjective sleep quality in human volunteers, correlating with polysomnographic changes. Yamadera et al., 2007

The purpose of this study was to examine the effects of glycine ingestion (3 g) before bedtime on subjective sleep quality, among people with poor sleep quality (Yamadera et al., 2007). A set of examinations over two consecutive nights and days was repeated twice with an interval at least 1 week between them. Changes in polysomnography during sleep were analysed. Effects on daytime sleepiness and daytime cognitive function were also evaluated. This was a randomised single-blind crossover trial of 11 volunteers.

A physician conducted a clinical interview (not more specifically described) for assessment of possible acute adverse effects. No serious acute adverse effects were observed.

2.4.1.2 Interactions

There was no information concerning interactions in the literature reviewed in the present risk assessment. The absence of information in the selected literature does not document an absence of interactions.

2.4.1.3 Allergic sensitisation (Including adjuvant effects)

There was no information concerning allergic sensitisation or allergy adjuvant effects in the literature reviewed in the present risk assessment. The absence of information in the selected literature does not document an absence of allergic sensitisation or allergy adjuvant effects.

2.4.2 Animal studies

A 4-week repeated dose toxicity study of glycine in rats by gavage administration. Shibui et al, 2013

The purpose of this study was to examine the toxicity profile of glycine. A solution of glycine in water for injection was administered orally (via gavage) to male rats once daily for 4 weeks at doses of 500, 1000 and 2000 mg/kg per day in a volume of 10 mL/kg (Shibui et al., 2013). Control animals received vehicle only. No animals died, and no glycine-related changes were observed in body weight, food consumption, water consumption, hematology, organ weight, gross pathological examination or histopathological examination. Some minor changes in urinary output were observed, but were considered to be of little toxicological significance as there were no histopathological changes in the kidneys or urinary bladder. As regards blood chemistry, phospholipids were significantly higher in the 2000 mg/kg per day dose group. However, the increase was small and was not considered to be toxicologically significant.

The authors concluded that none of the animals in any of the glycine-treated groups showed changes that were considered toxicologically significant. Therefore, the NOAEL was found to be at least 2000 mg/kg per day under the conditions of this study.

2.4.3 Mode of action for adverse effects

No mode of action for adverse effects has been identified.

2.4.4 Vulnerable groups

No vulnerable groups to excess doses of glycine have been reported. There have been no reported studies involving children, elderly, pregnant women or lactating women.

2.5 Summary of hazard identification and characterisation

Previous risk assessments did not conclude regarding safe doses of glycine due to lack of adequate scientific documentation. The IOM report from 2005 concluded that data on adverse effects of glycine intake from supplements were not sufficient for a dose-response assessment and derivation of a UL. The VKM report from 2011 concluded that no animal or human studies of glycine were found to be relevant for inclusion.

In the current report, VKM has not identified any human studies that were designed to evaluate potential harmful effects of glycine. Publications from three studies were identified that administered supplemental glycine to apparently healthy people. One of these reported results from two pilot studies in which 0.8 g glycine per day for up to 24 weeks was administered to people at risk of developing psychosis (Woods et al., 2013). No safety concerns were identified. Two Japanese studies of glycine supplementation, 3 g/day up to 4 days to improve sleep quality, did not find any adverse events (Inagawa et al., 2006; Yamadera et al., 2007).

None of these studies on humans provide evidence of adverse effects of glycine supplementation. They are, however, of limited value in order to evaluate potential adverse effects of glycine supplementation because they are of short duration and only to a limited extent have registered adverse effects.

Based on available data in humans it is not possible to establish a "value for comparison" for a risk characterisation of glycine. One study in rats (Shibui et al., 2013), estimated a NOAEL of glycine to be at least 2000 mg/kg per day. This was the highest dose tested.

VKM will use the NOAEL at 2000 mg/kg bw per day as a value for comparison in the risk characterisation of the specified doses in chapter 4.

3 Exposure / Intake

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

3.1 Food supplements

The Norwegian Food Safety Authority requested VKM to perform a risk assessment of 20, 50, 100, 300, 500 and 650 mg/day of glycine in food supplement for children (10 – 17 years) and adults. The default body weights for age groups determined by EFSA were used: 10 to <14 years = 43.4 kg, 14 to <18 years = 61.3 kg and adults = 70.0 kg. The exposures per kg bw are given in Table 3.1-1. All are below the estimated NOAEL given above of 20 mg per kg bw per day.

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

Groups	Daily doses (mg)	Body weight (kg)	Exposures (mg/kg bw per day)
Children (10 to <14 years)	20, 50, 100, 300, 500 and 650	43.4	0.5, 1, 2, 7, 12 and 15
Adolescents (14 to <18 years)	20, 50, 100, 300, 500 and 650	61.3	0.3, 1, 2, 5, 8 and 11
Adults (≥18 years)	20, 50, 100, 300, 500 and 650	70.0	0.3, 1, 1, 4, 7 and 9

3.2 Other sources

Rich food sources of glycine are protein-rich foods including meat, fish, dairy products and legumes. Glycine is also found in high amounts in gelatin, a substance made from collagen. Dietary sources of gelatin include gelatin desserts and gummy candy. It is also an additive in various food products, such as yogurt, cream cheese, margarine and ice cream.

Based on distribution data from the 1988–1994 NHANES III, the mean daily intake for all life stage and gender groups of glycine from food and supplements is 3.2 g/day. Men 19 through 30 years of age had the highest intakes at the 50th percentile of 4.6 g/day and at the 99th percentile of 7.8 g/day (IOM, 2005).

4 Risk characterisation

The doses received from NFSA for assessment were 20, 50, 100, 300, 500 and 650 mg/day glycine in food supplements, and the estimated exposures for adults, adolescents and children 10 years and older derived from these dose levels are given in chapter 3.

No human studies can be used for suggesting a "value for comparison", and there are no scientific data on human studies in the published literature suitable for assessing the specific doses in the terms of reference. Several studies investigating glycine at doses ranging from 0.8 to 3 g/day did not report any adverse effects. The uncertainties are described in chapter 5.

The NOAEL at 2000 mg/kg per day (highest dose tested) from an animal toxicity study in rats has been used to calculate the Margin of Exposure (MOE), the ratio of the NOAEL to the specified doses of glycine. The MOE-values are presented in Table 4-1.

Table 4-1: The calculated margins between the highest dose tested in humans not associated with adverse effect (2000 mg/kg bw per day) from an animal study and the exposure to the specified doses of glycine in food supplements (MOE-values) for the various age groups.

Age groups	20 mg/day	50 mg/day	100 mg/day	300 mg/day	500 mg/day	650 mg/day
Children (10 to <14 years) (43.4 kg)⁴	4340	1736	868	289	174	134
Adolescents (14 to <18 years) (61.3 kg)	6130	2452	1226	409	245	189
Adults (≥18 years) (70 kg)	7000	2800	1400	467	280	215

The MOE-values range from 134 to 7000. An uncertainty factor of 100 (10 for interspecies and 10 for inter individual variation) is applicable for all the doses in the three age groups. Using an uncertainty factor of 100, this corresponds to 20 mg/kg per day, or 1.4 g per day for a person weighing 70 kg.

Our literature review did not identify any studies of glycine in children or adolescents, and there were no studies in children 10 years or older included in previous risk assessments. However, there are no data indicating that children and adolescent are more vulnerable than adults for glycine. No tolerance level is set for glycine specifically for children or adolescents.

VKM considers that in adults (≥ 18 years), adolescents (14 to < 18 years) and children (10 to < 14 years), the specified doses 20, 50, 100, 300, 500 and 650 mg/day of glycine from food supplements are unlikely to cause adverse health effects.

5 Uncertainties

- A major uncertainty is the lack of studies in humans reporting on potential adverse health effects of glycine supplementation.
- Only one animal study, performed on rats, was found to meet our inclusion criteria in this risk assessment.

6 Conclusions with answers to the terms of reference

The Norwegian Food Safety Authority (NFSA) requested the Norwegian Scientific Committee for Food Safety (VKM) to assess the safety of glycine in food supplements at the doses 20, 50, 100, 300, 500 and 650 mg/day for the general population, ages 10 years and above.

No data on humans were available to suggest a "value for comparison" or make a risk characterisation for the specified doses of glycine. One study in rats found a NOAEL of 2000 mg/kg per day. Using an uncertainty factor of 100, this corresponds to 20 mg/kg per day, or 1.4 g per day for a person weighing 70 kg.

No particular vulnerable groups for glycine supplements have been identified.

VKM concludes that:

- In adults (≥ 18 years), the specified doses 20, 50, 100, 300, 500 and 650 mg/day of glycine from food supplements are unlikely to cause adverse health effects.
- In adolescents (14 to < 18 years), the specified doses 20, 50, 100, 300, 500 and 650 mg/day of glycine from food supplements are unlikely to cause adverse health effects.
- In children (10 to < 14 years), the specified doses 20, 50, 100, 300, 500 and 650 mg/day of glycine from food supplements are unlikely to cause adverse health effects.

An overview of the conclusions is presented in Table 6-1.

Table 6-1: An overview of the conclusions for glycine in food supplements.
Green: Estimated exposures to L-glycine are unlikely to cause adverse health effects.

Doses	Glycine					
	20 mg/day	50 mg/day	100 mg/day	300 mg/day	500 mg/day	650 mg/day
Age groups						
Children (10 to < 14 years)						
Adolescents (14 to < 18 years)						
Adults (≥ 18 years)						

7 Data gaps

- There are no reports concluding regarding negative health effects related to glycine in adults (> 18 years) children or adolescents.
- No studies are found that include effects of these substances in lactating or pregnant women.
- There is lack of an acute reference dose or other acute data for glycine.

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Appendix 1

Search strategies for this risk assessment

Search strategy human studies

Database: Embase <1974 to 2016 April 05>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present>

1. glycine*.ti. (26102)
2. (risk* or safety or adverse or side-effect*1 or hazard* or harm* or negative or contraindicat* or contra-indicat* or interact* or toxicity or toxic).tw. (9789711)
3. 1 and 2 (4463)
4. (conference abstract* or letter* or editorial*).pt. (4934471)
5. 3 not 4 (4293)
6. limit 5 to (danish or english or norwegian or swedish) (4165)
7. limit 6 to human (1052)
8. remove duplicates from 7 (637)

Search strategy animal studies

Database: Embase <1974 to 2016 August 01>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present>

1. glycine*.ti. (26512)
2. (risk* or safety or adverse or side-effect*1 or hazard* or harm* or negative or contraindicat* or contra-indicat* or interact* or toxicity or toxic).tw. (10134161)
3. 1 and 2 (4558)
4. (conference abstract* or letter* or editorial*).pt. (5120667)
5. 3 not 4 (4381)
6. limit 5 to (danish or english or norwegian or swedish) (4253)
7. limit 6 to animals (1766)
8. remove duplicates from 7 (1095)