EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) ; Scientific Opinion on Flavouring Group Evaluation 305 (FGE.305): L - Methionylglycine of chemical group 34

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 305 (FGE.305): *L*-Methionylglycine of chemical group 34[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate one flavouring substance, the dipeptide *L*-methionylglycine [FL-no: 17.037], in the Flavouring Group Evaluation 305, using the Procedure in Commission Regulation (EC) No 1565/2000. The substance was considered not to have genotoxic potential. The substance was evaluated through a stepwise approach (the Procedure) that integrates information on the structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel concluded that for the flavouring substance, evaluated through the Procedure, no appropriate NOAEL was available and additional data are required. The present evaluation of the candidate substance *L*-methionylglycine [FL-no: 17.037] is only applicable for its use in foods that are not heated or intended to be heated. Besides the safety assessment of the flavouring substance, the specifications for the material of commerce have also been considered. Adequate specifications including complete purity criteria and identity for the material of commerce have been provided for the candidate substance.

KEY WORDS

Flavourings, food safety, *L*-methionylglycine, dipeptide, amino acid

[1] On request from the European Commission, Question No EFSA-Q-2010-01503, adopted on 19 March 2013.

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SUMMARY

Following a request from the European Commission the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) was asked to deliver a scientific opinion on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to evaluate one flavouring substance, the dipeptide *L*-methionylglycine [FL-no: 17.037], in the Flavouring Group Evaluation 305 (FGE.305), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000[4]. This flavouring substance belongs to chemical group 34, Annex I of the Commission Regulation (EC) No 1565/2000.

The candidate substance possesses one chiral centre and the optical isomer has been specified.

The candidate substance belongs to structural class III and has not been reported to occur naturally in any food items according to TNO.

In its evaluation, the Panel as a default used the Maximised Survey-derived Daily Intake (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe. However, when the Panel examined the information provided by the European Flavouring Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach.

In the absence of more precise information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a modified Theoretical Added Maximum Daily Intake (mTAMDI) approach based on the normal use levels reported by Industry. In those cases where the mTAMDI approach indicated that the intake of a flavouring substance might exceed its corresponding threshold of concern, the Panel decided not to carry out a formal safety assessment using the Procedure. In these cases the Panel requires more precise data on use and use levels.

According to the default MSDI approach the intake in Europe for the candidate substance *L*-methionylglycine [FL-no: 17.037] is 1.2 µg/*capita*/day.

No data on genotoxicity has been submitted for the candidate substance. However, consideration of the chemical structure does not give rise to safety concern with respect to genotoxicity.

No information has been provided on hydrolysis of the candidate substance *L*-methionylglycine [FL-no: 17.037] under physiological conditions. Without information about the potential for hydrolysis of the candidate substance *L*-methionylglycine [FL-no: 17.037] and without any studies that show the fate of the substance *in vitro* and/or *in vivo*, it is not possible to predict whether it will be absorbed as a dipeptide or not, nor its distribution or potential bioactivity after absorption. Since such information is lacking, rapid metabolism of the dipeptide to innocuous metabolites cannot be anticipated. Therefore, evaluation of the candidate substance proceeds via the B-side to step B4 of the Procedure, at which step no adequate study from which a No Observed Adverse Effect Level (NOAEL) was available. So, the Panel concluded that additional data are required for the candidate substance *L*-methionylglycine [FL-no: 17.037].

[4] Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. Official Journal of the European Communities 19.7.2000, L 180, 8-16.

When the estimated intake was based on the mTAMDI, it was 24000 µg/person/day, which is above the threshold for structural class III of 90 µg/person/day.

The Panel noted the high discrepancy between MSDI and mTAMDI. The hypothetical nature of the MSDI, which is based on anticipated volumes of production, leads to a high uncertainty in the safety evaluation of this substance when based on the MSDI. The Panel will therefore not be in a position to conclude on the absence of safety concern for this specific substance unless a more refined dietary exposure estimate based on use levels is provided.

The Panel further noted that the nature of the candidate substance and the proposed intended uses indicated by Industry suggests that the candidate substance may be a flavour precursor. The present evaluation of the candidate substance *L*-methionylglycine [FL-no: 17.037] is only applicable for its use in foods that are not heated or intended to be heated.

Adequate specifications, including purity criteria and identity for the material of commerce, have been provided for the candidate substance.

For the candidate substance *L*-methionylglycine [FL-no: 17.037] additional data are required, as no adequate study was available from which a NOAEL could be established.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Regulation (EC) No 2232/96[5] of the European Parliament and the Council lays down a Procedure for the establishment of a list of flavouring substances the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC[6], as last amended by Commission Decision 2009/163/EC[7]. Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000, which is broadly based on the opinion of the Scientific Committee on Food (SCF, 1999a).

The Union list of flavourings and source materials is established in Commission Regulation (EC) No 872/2012[8].

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests the European Food Safety Authority EFSA to carry out a safety assessment on the flavouring substance *L*-methionylglycine [FL-no: 17.037], in accordance with Commission Regulation (EC) No 1565/2000.

[5] Regulation No 2232/96 of the European Parliament and of the Council of 28 October 1996. Official Journal of the European Communities 23.11.1996, L 299, 1-4.

[6] Commission Decision 1999/217/EC of 23 February 1999 adopting a register of flavouring substances used in or on foodstuffs. Official Journal of the European Communities 27.3.1999, L 84, 1-137.

[7] Commission Decision 2009/163/EC of 26 February 2009 amending Decision 1999/217/EC as regards the Register of flavouring substances used in or on foodstuffs. Official Journal of the European Union 27.2.2009, L 55, 41.

[8] Commission implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. Official Journal of the European Communities 2.10.2012, L 267, 1-161.

ASSESSMENT

1. Presentation of the Substances in Flavouring Group Evaluation 305

1.1. Description

The present Flavouring Group Evaluation 305 (FGE.305), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (the Procedure – shown in schematic form in Annex I of this FGE), deals with one flavouring substance from chemical group 34 of Annex I of Commission Regulation (EC) No 1565/2000.

The flavouring substance under consideration in the present evaluation, with its chemical Register name, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) numbers, structure and specifications is listed in Table 3.

The flavouring substance *L*-methionylglycine [FL-no: 17.037] (candidate substance) is a dipeptide consisting of the essential amino acid, *L*-methionine [FL-no: 17.027] (evaluated in FGE.26Rev1 (EFSA, 2008b)) and the non-essential amino acid glycine [FL-no: 17.034] (evaluated by the JECFA and supporting substance in FGE.26Rev1). If hydrolysed, the candidate substance will yield these two amino acids.

Industry has stated, in a communication of 22 February 2012 (Flavour Industry, 2012), that the dipeptide *L*-methionylglycine currently is not used as a flavouring substance, and that use figures for some food categories are estimates. This would imply that the production figure could change if/when the substance comes into use, and would further imply that the intake estimation using the MSDI approach, on which the current evaluation has been based, could be, or could become soon, superseded.

The Panel noted that amino acids may react with other food constituent upon heating. The reaction mixtures formed are commonly referred to as “process flavours”, which have not been evaluated by the Panel. The present evaluation is therefore carried out on the basis that the flavouring substance is used in foods that are not intended to be heated and that it is in an unchanged form when consumed in food. Industry has stated that in their opinion it is justified to assume that this dipeptide will not change during processing, based on the fact that flavouring substances that are added to e.g. dairy products that are sterilised, need to be “heat stable” (Flavour Industry, 2012). No documentation has been submitted to underpin this assumption. As Industry has informed that the candidate substance is used in e.g. bakery wares, processed vegetables, soups, savouries etc., i.e. foodstuffs that presumably are intended to be heated, the implication is that the present evaluation may not cover all aspects of the intended use of the candidate flavouring substance.

The evaluation of the candidate flavouring substance [FL-no: 17.037] is based on that it is not used in foods that are heated or are intended to be heated.

A summary of the safety evaluation is summarised in Table 4.

1.2. Stereoisomers

It is recognised that geometrical and optical isomers of substances may have different properties. Their flavour may be different, they may have different chemical properties resulting in possible variability in their absorption, distribution, metabolism, elimination and toxicity. Thus, information must be provided on the configuration of the flavouring substance, i.e. whether it is one of the

geometrical/optical isomers, or a defined mixture of stereoisomers. The available specifications of purity will be considered in order to determine whether the safety evaluation carried out for candidate substances for which stereoisomers may exist can be applied to the material of commerce. Flavouring substances with different configurations should have individual chemical names and codes (CAS number, FLAVIS number etc.).

The candidate substance *L*-methionylglycine [FL-no: 17.037] possesses one chiral centre. The optical isomer has been specified (Flavour Industry, 2010) (Table 3).

1.3. Natural Occurrence in Food

According to TNO, the candidate substance *L*-methionylglycine [FL-no: 17.037] has not been reported to occur naturally in any food items (TNO, 2010).

Industry has stated that *L*-methionylglycine has been identified in cheddar cheese (unpublished internal analysis, no quantitative data provided) (Flavour Industry, 2010) and in porcine heart (Guoliang et al., 1986). From the presentation of this study it is however not possible to assess the validity of the statement that *L*-methionylglycine should be naturally present in porcine heart.

2. Specifications

Purity criteria for the candidate substance have been provided by the Flavour Industry (Flavour Industry, 2010) (Table 3).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000, this information is adequate for the candidate substance (see Section 1.2 and Table 3).

3. Intake Data

Annual production volumes of the flavouring substances as surveyed by the Industry can be used to calculate the "Maximised Survey-derived Daily Intake" (MSDI) by assuming that the production figure only represents 60 % of the use in food due to underreporting and that 10 % of the total EU population are consumers (SCF, 1999).

However, the Panel noted that due to year-to-year variability in production volumes, to uncertainties in the underreporting correction factor and to uncertainties in the percentage of consumers, the reliability of intake estimates on the basis of the MSDI approach is difficult to assess.

The Panel also noted that in contrast to the generally low *per capita* intake figures estimated on the basis of this MSDI approach, in some cases the regular consumption of products flavoured at use levels reported by the Flavour Industry in the submissions would result in much higher intakes. In such cases, the human exposure thresholds below which exposures are not considered to present a safety concern might be exceeded.

Considering that the MSDI model may underestimate the intake of flavouring substances by certain groups of consumers, the SCF recommended also taking into account the results of other intake assessments (SCF, 1999).

One of the alternatives is the "Theoretical Added Maximum Daily Intake" (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake by most consumers because it is based on the

assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (e.g., it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004).

3.1. Estimated Daily per Capita Intake (MSDI Approach)

The intake estimation is based on the Maximised Survey-derived Daily Intake (MSDI) approach, which involves the acquisition of data on the amounts used in food as flavourings (SCF, 1999). These data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the population[9] (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999).

The anticipated total annual volume of production of the candidate substance in the present Flavouring Group Evaluation (FGE.305) from use as flavouring substance in Europe has been reported to be approximately 10 kg (Flavour Industry, 2010). On the basis of the anticipated annual volume of production reported for the candidate substance, the estimated intake of *L*-methionylglycine from use as a flavouring substance is 1.2 µg *per capita* per day (Table 4).

3.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI)

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995).

The assumption is that a person may consume a certain amount of flavourable foods and beverages per day.

For the candidate substance information on food categories and normal and maximum use levels[10,11] was submitted by the Flavour Industry (Flavour Industry, 2010). The candidate substance is proposed to be used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000, as shown in Table 1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

[9] EU figure 375 millions. This figure relates to EU population at the time for which production data are available, and is consistent (comparable) with evaluations conducted prior to the enlargement of the EU. No production data are available for the enlarged EU.

[10] "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002).

[11] The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004).

Table 1: Use of the Candidate Substance in Various Food Categories

Food category	Description	Flavouring used
01.0	Dairy products, excluding products of category 2	Yes
02.0	Fats and oils, and fat emulsions (type water-in-oil)	No
03.0	Edible ices, including sherbet and sorbet	No
04.1	Processed fruits	Yes
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Yes
05.0	Confectionery	No
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	No
07.0	Bakery wares	Yes
08.0	Meat and meat products, including poultry and game	No
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	No
10.0	Eggs and egg products	No
11.0	Sweeteners, including honey	No
12.0	Salts, spices, soups, sauces, salads, protein products etc.	Yes
13.0	Foodstuffs intended for particular nutritional uses	No
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	No
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	No
15.0	Ready-to-eat savouries	Yes
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	No

According to the Flavour Industry the normal use level for the candidate substance is in the range of 50 - 150 mg/kg food and the maximum use level is in the range of 1000 - 3000 mg/kg (Flavour Industry, 2010) (see Table II.1.2, Annex II). The mTAMDI value is 24000 µg/person/day (see Section 5).

4. Absorption, distribution, metabolism and elimination

No studies have been provided for the absorption, distribution, metabolism and excretion (ADME) of the candidate substance *L*-methionylglycine [FL-no: 17.037], a dipeptide. There is no information as to whether the dipeptide is absorbed intact or not, or regarding its fate or potential adverse biological activities if it is absorbed in intact form.

Di- and tripeptides may be absorbed rapidly and effectively from the intestinal canal, and transport of amino acids in the form of small peptides may be a faster route of uptake than that of the amino acids in free form. In humans, two di- and tripeptide transporters have been identified, human peptide transporter 1 and 2. Peptides may also be absorbed intact via other mechanisms such as by passive diffusion, paracellular route, endocytosis or carrier mediated transport. Proteins and peptides may be transported from the intestinal lumen to the blood circulation in biologically significant amounts. Di- and tripeptides are prone to be systemically distributed. Composition of dietary protein may affect the levels of circulating peptides. A study (Matthews and Webb, 1995) showed that the candidate substance *L*-methionylglycine [FL-no: 17.037] is transferred intact through two types of sheep epithelial tissue *in vitro*.

Dipeptides can be hydrolysed to component amino acids by several peptidases present in several organs, but rate and extent of hydrolysis may vary considerably, and there are great differences in the rate of hydrolysis of small peptides.

Research on oral availability of bioactive peptides is gaining attention, and several studies show that peptides may be absorbed intact with retained biological activity after oral administration. In a study on rats designed to determine the effect of amino acid chain length on the ability of enterally administered peptides to produce biological effects, the results showed that the shorter the amino acid chain length the more bioactivity was retained. E.g. the tripeptide thyrotropin releasing hormone had the same effect when administered enterally as when administered intravenously (Roberts et al., 1999).

If hydrolysed, the dipeptide *L*-methionylglycine will generate the two amino acids methionine and glycine, components of dietary protein. Methionine and glycine are metabolised to innocuous products when ingested at amounts that occur naturally in the diet. However, methionine can be converted via de-methylation to homocysteine. High intakes of single amino acids may lead to amino acid imbalances that may affect uptake, metabolism pathways and mechanisms of transport etc.

Without information about the potential for hydrolysis of the candidate substance *L*-methionylglycine [FL-no: 17.037] and without any studies that show the fate of the substance *in vitro* and/or *in vivo*, it is not possible to predict whether it will be absorbed as a dipeptide or not, nor its distribution or immediate fate after absorption, e.g. if there is any tissue specific availability or specificity for utilization of this dipeptide. Since such information is lacking, rapid metabolism of the dipeptide to innocuous metabolites cannot be anticipated.

For more detailed information, see Annex III.

5. Application of the Procedure for the Safety Evaluation of Flavouring Substances

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure. In these cases the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 6.

The present evaluation of the flavouring candidate substance *L*-methionylglycine [FL-no: 17.037] is based on the assumption that it is not used in foods that are heated or are intended to be heated.

Step 1

The candidate substance *L*-methionylglycine [FL-no: 17.037] is classified according to the decision tree approach by Cramer et al. into structural class III (Cramer et al., 1978).

Step 2

Step 2 requires consideration of the metabolism of the candidate substance. No information has been provided on hydrolysis of the candidate substance [FL-no: 17.037] under physiological conditions, Without information about the potential for hydrolysis of the candidate substance *L*-methionylglycine [FL-no: 17.037] and without any studies that show the fate of the substance *in vitro* and/or *in vivo*, it is not possible to predict whether it will be absorbed as a dipeptide or not, nor to its distribution or potential bioactivity after absorption. Since such information is lacking, rapid metabolism of the dipeptide to innocuous metabolites cannot be anticipated. The candidate substance will subsequently proceed via the B-side of the Procedure.

Step B3

The estimated daily *per capita* intake of the candidate substance [FL-no: 17.037] is 1.2 µg according to the MSDI approach. This is below the threshold for structural class III of 90 µg/person/day.

Step B4

No adequate study from which a NOAEL could be established was available. Therefore the Panel concluded that additional data are required for the candidate substance *L*-methionylglycine [FL-no: 17.037].

6. Comparison of the Intake Estimations Based on the MSDI Approach and the mTAMDI Approach

The estimated intake of the substance [FL-no: 17.037] assigned to structural class III, based on the mTAMDI, is 24000 µg/person/day, which is above the threshold of concern for structural class III of 90 µg/person/day. According to the Flavour Industry the normal use level for the candidate substance is in the range of 50 - 150 mg/kg food and the maximum use level is in the range of 1000 - 3000 mg/kg.

The Panel noted the large differences in the MSDI and mTAMDI figures. See Table 2.

Since the candidate substance has not yet come into use, the anticipated annual production volume for use as flavouring substance in Europe does not reflect the actual state of use of the candidate substance. The reported use levels for this substance indicate that the actual use may lead to high intake figures. This is reflected by the very large discrepancy between the intake estimations according to the MSDI and mTAMDI approach.

Table 2: Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
17.037	L-Methionylglycine	1.2	24000	Class III	90

7. Considerations of Combined Intakes from Use as Flavouring Substances

Because of structural similarities of candidate and supporting substances, it can be anticipated that many of the flavourings are metabolised through the same metabolic pathways and that the metabolites may affect the same target organs. Further, in case of combined exposure to structurally related flavourings, the pathways could be overloaded. Therefore, combined intake should be considered. As flavourings not included in this FGE may also be metabolised through the same pathways, the combined intake estimates presented here are only preliminary. Currently, the combined intake estimates are only based on MSDI exposure estimates, although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.

The total estimated combined daily *per capita* intake of structurally related flavourings is estimated by summing the MSDI for individual substances.

Considerations of combined intakes are not applicable in this evaluation.

8. Toxicity

General information and specific studies

Peptides as well as single amino acids may have adverse biological activities.

Bioactive peptides usually contain between 2 and 20 amino acids, these peptides may be derived from food proteins or may be produced by processing food or produced synthetically (Vermeirssen et al.,

2004). Bioactive peptides have lately gained much interest as dietary components with beneficial or detrimental health potentials, and as substances that may be used for biomedical applications and for development of medicinal drugs. Oral bioavailability of bioactive peptides may vary greatly. In general the shorter the amino acid chain, the greater the probability of the peptide being absorbed intact with retained bioactivity.

For di- and higher peptides selective toxicity may affect specific physiological processes. Zaloga and Siddiqui reviewed some dietary peptides with biological activity (Zaloga and Siddiqui, 2004). In this review it is stated that: "Importantly, many dietary peptides are biologically so potent that even small amounts entering the circulation could have major pathophysiological significance. These peptides may produce their affects in the body at concentrations of micrograms to milligrams per mL. Since human adults absorb approximately 100 g protein per day (i.e. 1 - 1.5 g/kg), only a small fraction of ingested protein need be absorbed [as biologically active protein or peptides] to produce systemic effects." Some examples of dietary peptides with bioactivity that are mentioned are peptides derived from casein or soy proteins that modulate immune function, e.g. methionine-enkephalin, a pentapeptide.

Among others, the following examples of bioactive peptides with retained oral activity may be mentioned. Pepsin digests from tuna inhibit angiotensin-I converting enzyme (ACE) and oral administration of digests significantly reduced blood pressure of rats. In this study (Astawan et al., 1995), four inhibitor peptides were found, two penta- and two hexapeptides ACE-inhibitory peptides may be produced in fermented milk, and two such tripeptides have been shown to be absorbed intact with retained bioactivity in rats and fermented milk containing the tripeptides had blood pressure reducing activity when administered orally to humans (Masuda et al., 1996; Seppo et al., 2003). The hypoglycemic dipeptide cyclo His-Pro, that may be found in different food items, has oral bioavailability with retained bioactivity (Hilton et al., 1990; Choi et al., 2013).

L-Methionylglycine [FL-no: 17.037]

For the candidate substance no reliable information has been made available or found in a literature search.

There is one *in vitro* study published on physiological effects of the candidate substance. The object was to look for endogenous peptides with activity on heart muscle. *L*-Methionylglycine was isolated from porcine heart (as referred in Section 1.3.). When this dipeptide was tested on cultured myocardial cells from rat, the beating rate of the cells increased about 30 % (Guoliang et al., 1986). However, from the presentation of the study it is not possible to assess the validity of these observations.

Methionine

Overload of a single amino acid, e.g. *L*-methionine, may lead to amino acid imbalance resulting in toxicity.

Any toxic effects of methionine may be accounted for by metabolites, i.e. homocysteine (Toue et al., 2006; Hanratty et al., 2001). Homocysteine has been implicated in cardiovascular, hepatic and cognitive disease (IOM, 2002).

Amino acid composition of the proteins may have effect on plasma cholesterol levels. Reports suggest that dietary proteins with low ratios of methionine-glycine and lysine-arginine favor a hypocholesterolemic effect, and that e.g. bovine casein tends to elevate plasma cholesterol levels due to its high ratios of methionine-glycine and lysine-arginine (Morita et al., 1997; Erdmann et al., 2008).

Daily dietary intake of methionine is approximately 1.4 g for a person consuming 100 g protein per day.

So far, in spite of efforts made, no upper level has been established for methionine due to lack of dose-response data. The ANS-panel considered an addition to the diet of 57.2 mg methionine/day, corresponding to about 0,95 mg methionine/kg body weight/day, to be negligible compared to the normal dietary intake (EFSA, 2008a). The mTAMDI value for *L*-methionylglycine [FL-no: 17.037] is 24 mg/person/day, as shown in Section 6. This corresponds to about 17.4 mg methionine.

Conclusion

Both single amino acids and peptides may have adverse biological activities. Some dietary peptides have great potency and may exert effects at concentrations ranging from µg – mg/mL plasma. If the candidate substance *L*-methionylglycine [FL-no: 17.037] is absorbed intact there is no data on the fate of the substance or its potential adverse biological activity and potency.

8.1. Acute Toxicity

No data are available for the candidate substance, *L*-methionylglycine [FL-no: 17.037].

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Subacute and subchronic toxicity data are not available for the candidate substance, *L*-methionylglycine [FL-no: 17.037].

8.3. Developmental / Reproductive Toxicity Studies

No data on developmental toxicity and reproductive toxicity are available for the candidate substance, *L*-methionylglycine [FL-no: 17.037].

8.4. Genotoxicity Studies

No *in vitro* or *in vivo* genotoxicity data are available for the candidate substance, *L*-methionylglycine [FL-no: 17.037].

Conclusion on Genotoxicity

No data on genotoxicity has been submitted for the candidate substance; however, consideration of the chemical structure does not give rise to safety concern with respect to genotoxicity.

CONCLUSIONS

The FGE.305 deals with the evaluation of one candidate substance, the dipeptide *L*-methionylglycine [FL-no: 17.037].

The candidate substance possesses one chiral centre and the optical isomer has been specified. It belongs to structural class III and has not been reported to occur naturally in any food items according to TNO.

According to the default MSDI approach the intake in Europe for the candidate substance [FL-no: 17.037] is 1.2 µg/*capita*/day.

No data on genotoxicity has been submitted for the candidate substance. However, consideration of the chemical structure does not give rise to safety concern with respect to genotoxicity.

No information has been provided on hydrolysis of the candidate substance [FL-no: 17.037] under physiological conditions. Without information about the potential for hydrolysis of the candidate substance *L*-methionylglycine [FL-no: 17.037] and without any studies that show the fate of the substance *in vitro* and/or *in vivo*, it is not possible to predict whether it will be absorbed as a dipeptide or not, nor its distribution or potential bioactivity after absorption. Since such information is lacking, rapid metabolism of the dipeptide to innocuous metabolites cannot be anticipated. Therefore, evaluation of the candidate substance proceeds via the B-side to step B4 of the Procedure, at which step no adequate study from which a No Observed Adverse Effect Level (NOAEL) was available. So, the Panel concluded that additional data are required for the candidate dipeptide *L*-methionylglycine [FL-no: 17.037].

When the estimated intake was based on the mTAMDI, it was 24000 µg/person/day, which is above the threshold for structural class III of 90 µg/person/day.

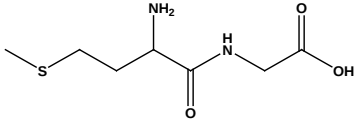
The Panel noted the high discrepancy between MSDI and mTAMDI. The hypothetical nature of the MSDI, which is based on anticipated volumes of production, leads to a high uncertainty in the safety evaluation of this substance when based on the MSDI. The Panel will therefore not be in a position to conclude on the absence of safety concern for this specific substance unless a more refined dietary exposure estimate based on use levels is provided.

The Panel further noted that the nature of the candidate substance and the proposed intended uses indicated by Industry suggests that the candidate substance may be a flavour precursor. The present evaluation of the candidate substance *L*-methionylglycine [FL-no: 17.037] is only applicable for its use in foods that are not heated or intended to be heated.

Adequate specifications, including purity criteria and identity for the material of commerce, have been provided for the candidate substance.

For the candidate substance *L*-methionylglycine [FL-no: 17.037] additional data are required, as no adequate study was available from which a NOAEL could be established.

Table 3: Specification Summary of the Substances in the Flavouring Group Evaluation 305

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
17.037	L-Methionylglycine		4692 14486-03-4	Solid $C_7H_{14}N_2O_3S$ 206.27	Soluble Soluble	 201 MS 98 %	n.a. n.a.	

1) *Solubility in water, if not otherwise stated.*

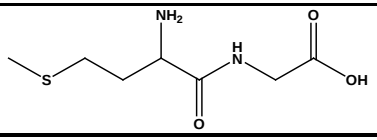
2) *Solubility in 95 % ethanol, if not otherwise stated.*

3) *At 1013.25 hPa, if not otherwise stated.*

4) *At 20°C, if not otherwise stated.*

5) *At 25°C, if not otherwise stated.*

Table 4: Summary of Safety Evaluation Applying the Procedure (Based on Intakes Calculated by the MSDI Approach)

FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
17.037	L-Methionylglycine		1.2	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required	6)	

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.

2) Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.

3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

4) No safety concern based on intake calculated by the MSDI approach of the named compound.

5) Data must be available on the substance or closely related substances to perform a safety evaluation.

6) No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 3 (based on intake calculated by the MSDI approach).

7) Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.

8) No conclusion can be drawn due to lack of information on the purity of the material of commerce.

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ANNEXES

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000, named the "Procedure", is shown in schematic form in Figure I.1. The Procedure is based on the opinion of the Scientific Committee on Food expressed on 2 December 1999 (SCF, 1999), which is derived from the evaluation Procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44th, 46th and 49th meetings (JECFA, 1995; JECFA, 1996; JECFA, 1997; JECFA, 1999).

The Procedure is a stepwise approach that integrates information on intake from current uses, structure-activity relationships, metabolism and, when needed, toxicity. One of the key elements in the Procedure is the subdivision of flavourings into three structural classes (I, II, III) for which thresholds of concern (human exposure thresholds) have been specified. Exposures below these thresholds are not considered to present a safety concern.

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The thresholds of concern for these structural classes of 1800, 540 or 90 µg/person/day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996).

In Step 1 of the Procedure, the flavourings are assigned to one of the structural classes. The further steps address the following questions:

- can the flavourings be predicted to be metabolised to innocuous products[12] (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous[13] (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

In addition to the data provided for the flavouring substances to be evaluated (candidate substances), toxicological background information available for compounds structurally related to the candidate substances is considered (supporting substances), in order to assure that these data are consistent with the results obtained after application of the Procedure.

The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity. Therefore, the right is reserved to use alternative approaches if data on specific flavourings warranted such actions.

[12] "Innocuous metabolic products": Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent" (JECFA, 1997).

[13] "Endogenous substances": Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997).

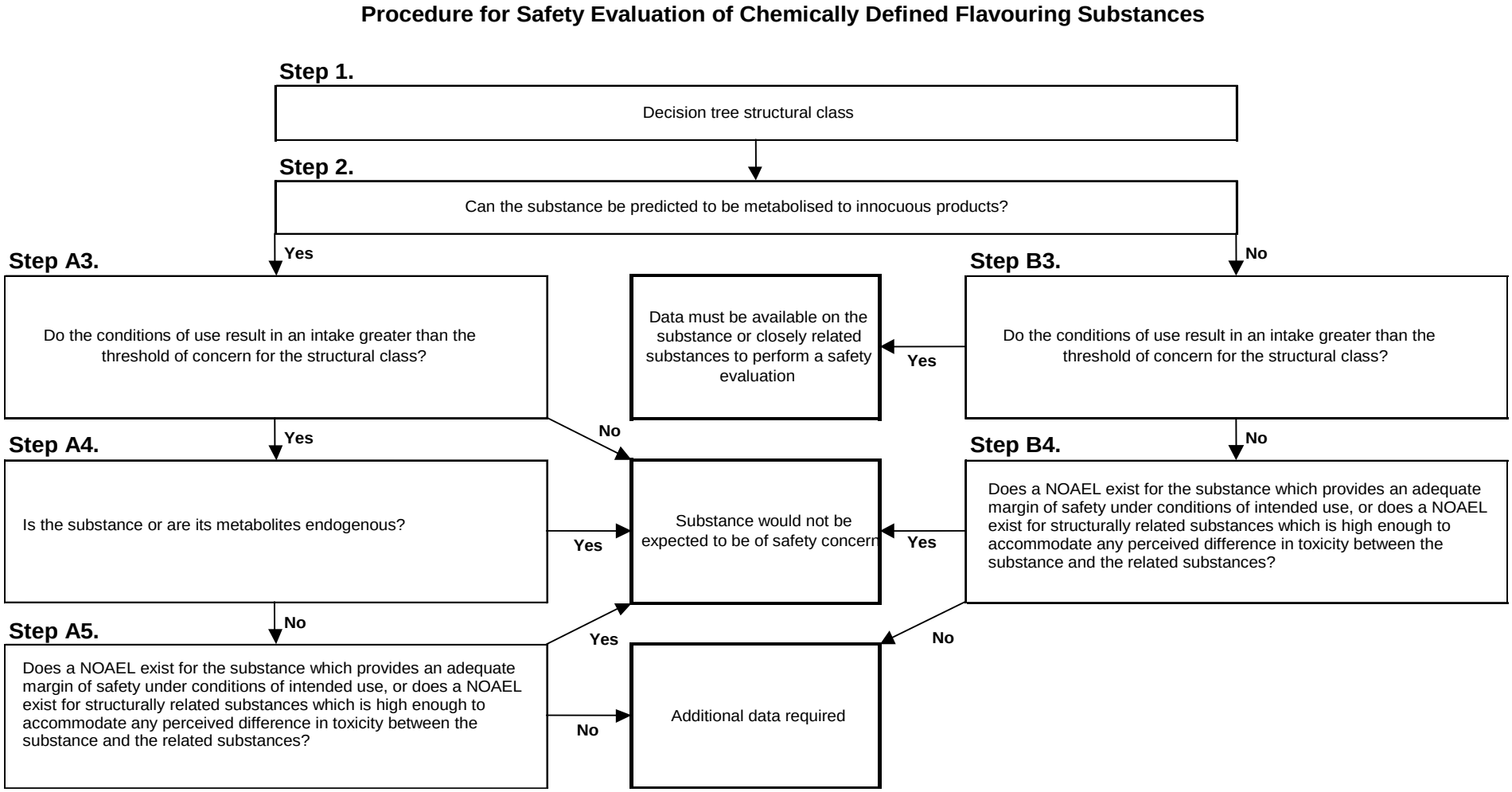


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

ANNEX II: USE LEVELS / MTAMDI

II.1 Normal and Maximum Use Levels

For each of the 18 Food categories (Table II.1.1) in which the candidate substance is used, Flavour Industry reports a “normal use level” and a “maximum use level”. According to the Industry the ”normal use” is defined as the average of reported usages and ”maximum use” is defined as the 95th percentile of reported usages (EFFA, 2002). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004).

Table II.1.1 Food categories according to Commission Regulation (EC) No 1565/2000

Food category	Description
01.0	Dairy products, excluding products of category 02.0
02.0	Fats and oils, and fat emulsions (type water-in-oil)
03.0	Edible ices, including sherbet and sorbet
04.1	Processed fruit
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds
05.0	Confectionery
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery
07.0	Bakery wares
08.0	Meat and meat products, including poultry and game
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms
10.0	Eggs and egg products
11.0	Sweeteners, including honey
12.0	Salts, spices, soups, sauces, salads, protein products, etc.
13.0	Foodstuffs intended for particular nutritional uses
14.1	Non-alcoholic ("soft") beverages, excl. dairy products
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts
15.0	Ready-to-eat savouries
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0

The “normal and maximum use levels” are provided by Industry (Flavour Industry, 2010) for the candidate substance in the present flavouring group (Table II.1.2).

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.305 (Flavour Industry, 2010).

FL-no	Food Categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	**02.0**	**03.0**	**04.1**	**04.2**	**05.0**	**06.0**	**07.0**	**08.0**	**09.0**	**10.0**	**11.0**	**12.0**	**13.0**	**14.1**	**14.2**	**15.0**	**16.0**
17.037	150	-	-	50	50	-	-	50	-	-	-	-	100	-	-	-	100	-
	3000	-	-	1000	1000	-	-	1000	-	-	-	-	2000	-	-	-	2000	-

II.2 mTAMDI Calculations

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table II.2.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table II.2.1 Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (g/day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: Candy, confectionery	27.0
Exception b: Condiments, seasonings	20.0
Exception c: Alcoholic beverages	20.0
Exception d: Soups, savouries	20.0
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)

The mTAMDI calculations are based on the normal use levels reported by Industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 and reported by the Flavour Industry in the following way (see Table II.2.2):

- Beverages (SCF, 1995) correspond to food category 14.1
- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13, and/or 16
- Exception a (SCF, 1995) corresponds to food category 5 and 11
- Exception b (SCF, 1995) corresponds to food category 15
- Exception c (SCF, 1995) corresponds to food category 14.2
- Exception d (SCF, 1995) corresponds to food category 12
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

Table II.2.2 Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

	Food categories according to Commission Regulation (EC) No1565/2000	Distribution of the seven SCF food categories		
Key	Food category	Food	Beverages	Exceptions
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excl. dairy products		Beverages	

Table II.2.2 Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

	Food categories according to Commission Regulation (EC) No1565/2000	Distribution of the seven SCF food categories	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts		Exception c
15.0	Ready-to-eat savouries		Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0	Food	

The mTAMDI value (see Table II.2.3) is presented for the flavouring substance in the present flavouring group, for which Industry has provided use and use levels (Flavour Industry, 2010). The mTAMDI value is only given for the highest reported normal use level.

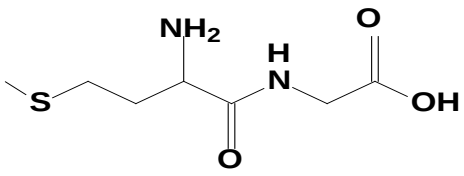
TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
17.037	L-Methionylglycine	24000	Class III	90

ANNEX III: METABOLISM

III.1. Introduction

The present FGE consists of one candidate substance, a dipeptide, *L*-methionylglycine [FL-no: 17.037].



L-Methionine and glycine belong to the group of twenty amino acids which are normal components of food proteins. *L*-Methionine is considered an essential amino acid for humans since it has to be provided by the diet and cannot be synthesised within the body. Protein intake in humans in the western world is estimated to be around 100 g/person/day (1 - 1.5 g/kg body weight) (Zaloga and Siddiqui, 2004). The requirement for methionine in the diet for mammals is considered to be 0.5 - 0.6 % of the diet (Yamada et al., 2012). For humans, dietary requirement for sulphur containing amino acids is 13 - 16 mg/kg body weight (bw)/day equivalent to 17 - 27 mg/g protein, but how much of this that can be methionine relative to cysteine is still controversial. Glycine is a non-essential amino acid, and out of the 20 amino acids that are building blocks of proteins, it is the only one that does not contain an asymmetrical carbon. The dipeptide *L*-methionylglycine [FL-no: 17.037] is however not reported to occur naturally in any food items according to TNO (TNO, 2010). According to an unpublished internal analysis with no quantitative data provided, Industry has informed that *L*-methionylglycine has been identified in cheddar cheese (Flavour Industry, 2010), and also in porcine heart (Guoliang et al., 1986), the validity of this study is however not possible to assess due to limitations in the study report, and lack of information on study design and execution.

III.2. Absorption, Distribution and Elimination

No studies on absorption, distribution or elimination of the candidate substance have been provided.

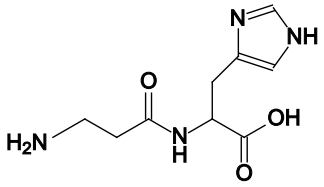
Proteins are digested in the low pH of the stomach by pepsin. In the more alkaline pH of the small intestine, pancreatic proteases further digest and cleave proteins and polypeptides resulting in oligopeptides and to a lesser extent of free amino acids. Free amino acids are absorbed into enterocytes via amino acid transport systems. Oligopeptides are further hydrolysed via brush border peptidases, mainly resulting in free amino acids and di- and tripeptides. Single amino acids are directly available for absorption by the small intestine and appear in the portal vein more rapidly than amino acids that are parts of proteins, e.g. intake of protein hydrolysates increased plasma levels of Val-Leu to a significantly greater extent than did ingestion of whole protein (Morifuji et al., 2010). Di- and tripeptides may be absorbed rapidly and effectively in the intestinal canal and transport of amino acids in the form of small peptides may be a faster route of uptake than that of the amino acids in free form (Webb, 1990; Erdmann et al., 2008). In humans, two di- and tripeptide transporters have been identified, human peptide transporter 1 and 2 (PepT1 and PepT2). The peptide transporter PepT1 can potentially transport all 400 dipeptides that may result as combinations of the 20 dietary amino acids. Di- and tripeptides have the capability to be, and may be prone to be, systematically distributed and may reach specific organs and tissues by transport via PepT1 and 2 (Santos et al., 2012). Peptides may also be absorbed intact via other mechanisms such as by passive diffusion, via paracellular route, via endocytosis or via carrier mediated transport (Vermeirssen et al., 2004). Intact proteins and peptides may be transported from the intestinal lumen to the blood circulation in biologically significant amounts (Gardner, 1988; Vermeirssen et al., 2004). According to Grimble (1994) as cited

by Zaloga and Siddiqui (Zaloga and Siddiqui, 2004) - 20 % of plasma amino acids are present as peptides.

Dipeptides can be hydrolysed by several peptidases present in several organs, but rate and extent of hydrolysis may vary greatly. As an illustration, in a study from 1960 (Johnston and Wiggans, 1960) the enzymatic breakdown of 15 peptides during 8 - 120 minutes was studied using peritoneal fluid from rats and humans, and a wide range of specificity was observed. Whereas e.g. *L*-leucyl-*L*-alanine was extensively hydrolysed after 8 minutes, e.g. glycylglycine was only slightly hydrolysed after 120 minutes, which was the length of the study. This study indicates great differences in the rate of hydrolysis of small peptides.

Data suggests that diet influences concentrations of circulating dipeptides and their availability to extrahepatic tissues. Tissue selectivity for peptide removal has been shown, which suggests that there may be tissue specific abilities to utilize circulating plasma peptides (Gilbert et al., 2008). For *L*-methionine-containing peptides the molecular structure influences the availability of the essential amino acid methionine. Methionine-containing peptides were utilized differently by three different cell types, implicating that there may be cell-specific differences in transport of small peptides, as well as in hydrolytic events (Gilbert et al., 2008; Pan and Webb, 1998).

The knowledge that some peptides are resistant to hydrolysis, and the finding of a peptide transporter in the basolateral membrane of enterocytes suggests that there may be a carrier-mediated mechanism for transport of peptides to the bloodstream (Terada et al., 1999). In mice the uptake of carnosine (*beta*-alaninehistidine), a dipeptide found in high amounts in muscle tissue, was found to be equally stimulated by high dietary levels of amino acids, peptides or proteins. Hydrolysis of carnosine was low or negligible (Ferraris et al., 1988).



Carnosine (*beta*-alaninehistidine)

Bioactive peptides are peptides, which may be derived from food proteins, are inactive when contained in the original protein, but have bioactivity as peptides. Bioactive peptides usually contain between 2 and 20 amino acids (Vermeirssen et al., 2004). Bioactive peptides have lately gained much interest as dietary components with beneficial or detrimental health potentials, as well as for biomedical applications and for development of medicinal drugs. An effect of this interest is that research on oral availability of bioactive peptides is gaining attention. In general, even though larger peptides may be absorbed orally and retain biological effects the potency of peptides decreases as the chain length increases (Erdmann et al., 2008). E.g. in a study designed to determine the effect of amino acid chain length on the ability of enterally administered peptides to produce biological effects, rats were administered biologically active peptides enterally and intravenously. The administered amount was less than 0.5 % of a rat's normal daily protein intake. The results indicated that 125 and 500 µg enteral administered thyrotropin-releasing hormone (a tripeptide) produced the same thyroid stimulating hormone-effect as when administered intravenously, the response of follicle stimulating hormone to 500 µg enteral luteinizing hormone-releasing hormone (a decapeptide) was 50 % of the same hormone administered intravenously, and that the glucose response to 25 mg enteral insulin (a 51-amino acid peptide) was 30 % of the response to 0.5 mg intravenous insulin. Both 0.5 and 25 mg enteral insulin significantly increased serum insulin levels (Roberts et al., 1999).

Angiotensin I converting enzyme (ACE) has a role in regulating blood pressure as it converts angiotensin I to angiotensin II, which is a potent vasoconstrictor, it also inactivates the vasodilator bradykinin. ACE inhibitors are used as antihypertensive drugs. Lately, different food proteins have

been identified as sources of ACE-inhibitory peptides, and there is on-going research on bioavailability and effectiveness of some of these peptides. Most ACE-inhibitory peptides consist of 2 - 9 amino acids. Two ACE-inhibitory tripeptides with blood pressure lowering effects (Val-Pro-Pro and Ile-Pro-Pro) produced by fermentation of milk by *Lactobacillus helveticus* and *Saccharomyces cerevisiae* (Calpis sour milk) were shown to decrease ACE activity and to be present in the aorta after a single oral administration of Calpis sour milk to spontaneously hypertensive rats (Masuda et al., 1996). In a 21 weeks study, hypertensive human subjects received 150 mL per day of either milk fermented by *Lactobacillus helveticus* standardised to contain the ACE-inhibitory peptides Val-Pro-Pro (2 mg/100 g product) and Ile-Pro-Pro (1.5 mg/100 g product) or a control milk fermented by a normal fermentation process with a *Lactococcus sp.* mixed culture. There was a mean difference of 6.7 + 3.0 mmHg in systolic blood pressure and of 3.6 + 1.9 in diastolic blood pressure between test product group and control group, indicating that the bioactive ACE-inhibiting peptides were absorbed intact. Other factors that might contribute to the blood pressure lowering effect might have been higher calcium content of test product compared to control and inclusion of live starter bacteria in the test product (Seppo et al., 2003). Oral bioavailability of bioactive peptides and difficulties to assess how much of a bioactive peptide that is absorbed intact, is a problem when using peptides for biomedical purposes and in drug development, as absorption of intact peptides may vary greatly. However, as the above examples show, small bioactive peptides may be absorbed intact and may exert biological activity.

L-Methionylglycine

In a search in the published literature not much data may be found concerning the candidate substance. *L*-Methionylglycine was shown to be transferred intact through two types of sheep epithelial tissue (Matthews and Webb, 1995), suggesting that the dipeptide has a potential to be absorbed in intact form.

Dipeptides with *L*-methionine at the *N*-terminal were shown to be utilised as a methionine source by cultured cells to a greater extent than peptides with *L*-methionine at the C-terminal (Pan et al., 1996).

Without information about the potential for hydrolysis of the candidate substance and lacking studies that show the fate of the substance *in vitro* or *in vivo*, it is not possible to predict whether it will be absorbed as a dipeptide or not, nor its possible fate if absorbed as a dipeptide.

Peptides administered orally may be absorbed intact and may have bioactivity, in general the longer the amino acid chain is the more of inherent bioactivity is lost during absorption. Small peptides such as di- and tripeptides are prone to be absorbed intact and distributed systemically.

In healthy adults, concentrations of amino acids in plasma are maintained in a relatively steady manner. In general, ingestion of single amino acids may create a transient imbalance of amino acids in the systemic circulation. Excess amino acids may lead to adverse effects due to imbalance of amino acid-status or antagonism among amino acids. Antagonism may occur among amino acids that are related, structurally or chemically.

III.3. Metabolism

If absorbed as such, there is no information on the specific fate of the dipeptide *L*-methionylglycine, whether the dipeptide itself exerts any specific adverse biological activity or not, nor if there is any tissue specific availability or specificity for utilization of this dipeptide. In an article that Industry has provided on natural occurrence of *L*-methionylglycine (Guoliang et al., 1986) it is stated that the dipeptide occurs in porcine heart, and also that the dipeptide has an effect on rat myocardial cells *in vitro* by increasing the beating rate by about 30 %. However, from the presentation of the study it is

not possible to evaluate the validity of the study, neither as to the natural presence of the dipeptide nor to its potential cardioactivity.

Glycine

Metabolism of glycine is closely linked to that of serine. The major pathway for glycine catabolism is catalysed by glycine cleavage complex in liver mitochondria:

Glycine + tetrahydrofolate + NAD^+ → CO_2 + NH_4^+ + N^5,N^{10}-Methylene tetrahydrofolate + NADH + H^+

Glycine may also be converted by transamination or oxidative deamination to glyoxylic acid, which is further metabolised oxalate and to formic acid. Another pathway for glycine is to be transformed to serine through a reversible reaction catalysed by serine hydroxymethyltransferase. *L*-Serine formed by this reaction may then form pyruvate and subsequently acetyl-CoA.

Glycine can be used to synthesise active one-carbon units, which are fragments activated by binding to tetrahydrofolic acid, or more seldom to thiamine pyrophosphate. Since glycine is one of the amino acids that participate in one-carbon metabolism and through this to methylation of proteins and DNA, it thereby participates in regulation of gene expression and biological activity of proteins (Wu, 2009).

L-Methionine

Methionine may be degraded via demethylation and transsulphuration or via transamination.

Methylation and transsulphuration pathway:

↗ transfer of –CH3 to other compounds

Methionine + ATP → *S*-Adenosylmethionine + PPi + Pi → *S*-Adenosylhomocysteine →

H2O ↘ ↗ Adenosine Serine↘ ↗H2O H2O↘

→ **Homocysteine** → **Cystathione** → **Cysteine + α-Ketobutyrate**

The initial step is activation to *S*-adenosylmethionine via ATP. *S*-Adenosylmethionine is a major donor of methyl groups, which with loss of the methyl group is converted to *S*-adenosylhomocysteine and sequently metabolised to homocysteine, combined with serine to yield cystathionine which may undergo further metabolism to cysteine, ammonia and *alpha*-ketobutyrate.

L-Cysteine can form taurine and CO_2, or sulphate, urea and CO_2. *alpha*-Ketobutyrate undergoes oxidative decarboxylation to propionyl-CoA, which through carboxylation yields *D*-methyl-malonyl-CoA and subsequently *L*-methyl-malonyl-CoA, which is rearranged to succinyl-CoA.

Homocysteine may be recycled back to methionine, which requires a folate derivative. Thus, folate deficiency may lead to a build-up of homocysteine.

Transamination pathway:

L-Methionine may be transaminated to *alpha*-keto-*gamma*-methylthiolbutyrate and then decarboxylated to 3-methylthioproprionyl CoA. From this the methylthiol moiety is cleaved to form methanethiol, which is subsequently metabolised to CO_2 and sulphate.

III.4. Summary and Conclusions

Studies have not been provided for the ADME of the candidate substance *L*-methionylglycine [FL-no: 17.037], a dipeptide.

If hydrolysed, the candidate substance will generate the two amino acids *L*-methionine and glycine, components of dietary protein. *L*-Methionine and glycine are metabolised to innocuous products at amounts that occur naturally in the diet. However, high intakes of single amino acids, may lead to amino acid imbalances and antagonism that may affect uptake, metabolism pathways and mechanisms of transport etc. The toxicity of *L*-methionine is mediated via the metabolite homocysteine.

Peptides administered orally may be absorbed intact and have bioactivity, in general the longer the amino acid chain is the more bioactivity is lost during absorption, or conversely the shorter the amino acid chain the greater the possibility that bioactivity is retained. Without information about the potential for hydrolysis of the candidate substance, and without any studies that show the fate of the substance *in vitro* or *in vivo*, it is not possible to predict whether it will be absorbed as a dipeptide or not, nor its distribution or immediate fate after absorption, e.g. if there is any tissue specific availability or specificity for utilization of this dipeptide. Since such information is lacking, rapid metabolism of the dipeptide to innocuous metabolites cannot be anticipated.

ABBREVIATIONS

ACE	Angiotensin I converting enzyme
ADI	Acceptable Daily Intake
ADME	Absorption, Distribution, Metabolism, and Excretion
ANS	Additives and Nutrient Sources
ATP	Adenosintriphosfat
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids Chemical Abstract Service
CHO	Chinese hamster ovary (cells)
CoA	Coenzyme A
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EC	European Commission
EFFA	European Flavour and Fragrance Association
EFSA	The European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
ID	Identity
IOFI	International Organization of the Flavour Industry
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50 %; Median lethal dose
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NAD	Nicotinamide Adenine Dinucleotide
NADH	Reduced Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
No	Number
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level

NTP	National Toxicology Program
PepT1	Peptide Transporter 1
Pi	Inorganic Phosphate
PPi	Inorganic Pyrophosphate
SCE	Sister Chromatid Exchange
SCF	Scientific Committee on Food
SMART	Somatic Mutation and Recombination Test
TAMDI	Theoretical Added Maximum Daily Intake
UDS	Unscheduled DNA Synthesis
WHO	World Health Organisation