European Food Safety Authority

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SCIENTIFIC OPINION

Flavouring Group Evaluation 79, (FGE.79)[1]

Consideration of amino acids and related substances evaluated by JECFA (63rd meeting) structurally related to amino acids from chemical group 34 evaluated by EFSA in FGE.26Rev1 (2008)

Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food

(Question No EFSA-Q-2008-063)

Adopted on 29 November 2007

PANEL MEMBERS

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SUMMARY

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (the Panel) is asked to advise the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular the Scientific Panel is requested to consider the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) evaluations of flavouring substances assessed since 2000, and to decide whether further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217/EC and its consecutive amendments.

The present consideration concerns 19 amino acids and related substances evaluated by the JECFA (63rd meeting) and will be considered in relation to the European Food Safety Authority (EFSA) evaluation of nine structurally related amino acids from chemical group 34 evaluated by EFSA in Flavouring Group Evaluation 26, Revision 1 (FGE.26Rev1).

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The Panel agrees with the evaluation performed by the JECFA for the 19 substances considered in this FGE.

The Panel noted that amino acids may react with other food constituents 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 on the basis that the flavouring substances in question are in an unchanged form when they are consumed, namely, in food that is not intended to be heated.

For five substances [FL-no: 16.056, 17.001, 17.003, 17.015 and 17.026] the JECFA evaluation is only based on Maximised Survey-derived Daily Intake (MSDI) values derived from production figures from the USA. EU production figures are needed in order to finalise the evaluation of these substances.

For all substances use levels are needed to calculate the modified Theoretical Added Maximum Daily Intake (mTAMDI) in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the 19 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications:

Specifications, including complete purity criteria and identity tests, are available for all the 19 JECFA evaluated substances.

Thus, the Panel concluded that the use of the Procedure was inappropriate for nine L-amino acids, glycine and one α-imino acid [FL-no: 17.003, 17.005, 17.007, 17.008, 17.012, 17.018, 17.019, 17.022, 17.026, 17.033 and 17.034] as the human exposures through food are in orders of magnitude higher than the anticipated levels of exposure from the use as flavouring substances. Therefore, these flavouring substances are not taken through the Procedure. However, the Panel concluded that nine of the substances were not of safety concern at their estimated levels of intake as flavouring substances. For two substances [FL-no: 17.003 and 17.026] no European production figures were available and the evaluation could not be finalised for these.

For three of the eight JECFA evaluated amino acids and related substances (using the Procedure) [FL-no: 16.056, 17.001 and 17.015] the Panel has reservations (no European production volumes available, preventing evaluation using the Procedure). For the remaining five substances [FL-no: 17.010, 17.014, 17.017, 17.023 and 17.024] the Panel agrees with the JECFA conclusion "no safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach.

KEYWORDS

Amino acids, chemical group 34, JECFA, FGE.26.

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996) 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 (EC, 1999a), as last amended by Commission Decision 2006/252/EC (EC, 2006). 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 (EC, 2000), which is broadly based on the opinion of the Scientific Committee on Food (SCF, 1999).

Commission Regulation (EC) No 1565/2000 lays down that substances that are contained in the Register and will be classified in the future by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) so as to present no safety concern at current levels of intake will be considered by the European Food Safety Authority (EFSA), who may then decide that no further evaluation is necessary.

In the period 2000 – 2006, during its 55th, 57th, 59th, 61st, 63rd and 65th meetings, the JECFA evaluated about 900 substances which are in the EU Register.

TERMS OF REFERENCE

EFSA is requested to consider the JECFA evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000). These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217 EC (EC, 1999a) and its consecutive amendments.

ACKNOWLEDGEMENTS

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ASSESSMENT

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000), hereafter named the "EFSA Procedure". This Procedure is based on the Opinion of the Scientific Committee on Food (SCF, 1999), which has been derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b), hereafter named the "JECFA Procedure". The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (the Panel) compares the JECFA evaluation of structurally related substances with the result of a corresponding EFSA evaluation, focussing on specifications, intake estimations and toxicity data, especially genotoxicity data. The evaluations by EFSA will conclude whether the flavouring substances are of no safety concern at their estimated levels of intake, whether additional data are required or whether certain substances should not be evaluated through the EFSA Procedure.

The following issues are of special importance.

Intake

In its evaluation, the Panel as a default uses the Maximised Survey-derived Daily Intake (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe.

In its evaluation, the JECFA includes intake estimates based on the MSDI approach derived from both European and USA production figures. The highest of the two MSDI figures is used in the evaluation by the JECFA. It is noted that in several cases, only the MSDI figures from the USA were available, meaning that certain flavouring substances have been evaluated by JECFA only on the basis of these figures. For Register substances for which this is the case the Panel will need EU production figures in order to finalise the evaluation.

When the Panel examined the information provided by the European Flavour 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. It is noted that the JECFA, at its 65th meeting, considered "how to improve the identification and assessment of flavouring agents, for which the MSDI estimates may be substantially lower than the dietary exposures that would be estimated from the anticipated average use levels in foods" (JECFA, 2006c).

In the absence of more accurate 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.

As information on use levels for the flavouring substances has not been requested by the JECFA or has not otherwise been provided to the Panel, it is not possible to estimate the daily intakes using the mTAMDI approach for the substances evaluated by the JECFA. The Panel will need information on use levels in order to finalise the evaluation.

Threshold of 1.5 Microgram/Person/Day (Step B5) Used by the JECFA

The JECFA uses the threshold of concern of 1.5 microgram/person/day as part of the evaluation procedure:

"The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 microgram per person per day would not be expected to present a safety concern. The Committee recommended that the Procedure for the Safety Evaluation of Flavouring Agents used at the forty-sixth meeting be amended to include the last step on the right-hand side of the original procedure ("Do the condition of use result in an intake greater than 1.5 microgram per day?")" (JECFA, 1999b).

In line with the Opinion expressed by the Scientific Committee on Food (SCF, 1999), the Panel does not make use of this threshold of 1.5 microgram per person per day.

Genotoxicity

As reflected in the Opinion of SCF (SCF, 1999), the Panel has in its evaluation focussed on a possible genotoxic potential of the flavouring substances or of structurally related substances. Generally, substances for which the Panel has concluded that there is an indication of genotoxic potential *in vitro,* will not be evaluated using the EFSA Procedure until further genotoxicity data are provided. Substances for which a genotoxic potential *in vivo* has been concluded will not be evaluated through the Procedure.

Specifications

Regarding specifications, the evaluation by the Panel could lead to a different opinion than that of the JECFA, since the Panel requests information on e.g. isomerism.

Structural Relationship

In the consideration of the JECFA evaluated substances, the Panel will examine the structural relationship and metabolism features of the substances within the flavouring group and compare this with the corresponding FGE.

1. Presentation of the Substances in the JECFA Flavouring Group

1.1. Description

1.1.1. JECFA Status

The JECFA has evaluated a group of 20 flavouring substances consisting of amino acids and related substances. The present consideration covers 19 substances as one substance (L-glutamic acid, JECFA-no: 1420) is not in the Register.

1.1.2. EFSA Considerations

The Panel concluded that the 19 substances in the JECFA flavouring group of amino acids and related substances are structurally related to the group of amino acids in chemical group 34 evaluated by EFSA in the Flavouring Group Evaluation 26, Revision 1 (FGE.26Rev1).

1.2. Isomers

1.2.1. JECFA Status

Sixteen of the substances have a chiral centre [FL-no: 17.003, 17.005, 17.007, 17.008, 17.010, 17.012, 17.014, 17.015, 17.017, 17.018, 17.019, 17.022, 17.023, 17.024, 17.026 and 17.033].

1.2.2. EFSA Considerations

No comments.

1.3. Specifications

1.3.1. JECFA Status

The JECFA specifications are available for all 19 substances (JECFA, 2005c). See Table 1.

1.3.2. EFSA Considerations

Specifications are considered adequate for all substances.

2. Intake Estimations

2.1. JECFA Status

For 14 of the 19 substances evaluated by the JECFA intake data are available for the EU, see Table 3.1. For the five remaining substances production figures are only available for the USA.

2.2. EFSA Considerations

As production figures are only available for the USA for five substances MSDI values for the EU cannot be calculated for these [FL-no: 16.056, 17.001, 17.003, 17.015 and 17.026].

3. Genotoxicity Data

3.1. Genotoxicity Studies – Text Taken from JECFA (JECFA, 2007a)

In vitro

In the assay for reverse mutation in *Salmonella typhimurium*, negative results were reported for glycine [FL-no: 17.034], methionine [FL-no: 17.014] and L-proline [FL-no: 17.019] at concentrations of up to 1000 mg/ml, with and without metabolic activation, in several strains of *S. typhimurium* (TA92, TA97, TA98, TA100, TA102, TA1530, TA1531, TA1532, TA1535, TA1537, TA1538 and TA1964) (Green & Savage, 1978; Baker & Bonin, 1981; Brooks & Dean, 1981; Ichinotsubo et al., 1981a; MacDonald, 1981; Nagao & Takahashi, 1981; Richold & Jones, 1981; Rowland & Severn, 1981; Trueman, 1981; Venitt & Crofton-Sleigh, 1981; Haworth et al., 1983; Fujita et al., 1994).

When evaluated in various strains of *Escherichia coli*, methionine [FL-no: 17.014] at concentrations of up to 6000 µg/plate (or 1000000 µg/ml), with or without metabolic activation did not show any evidence of mutagenic activity (Fluck et al., 1976; Ichinotsubo et al., 1981b; Matsushima et al., 1981; Mohn et al., 1981; Venitt & Crofton-Sleigh, 1981). When

E. coli WP2 *uvr*A was incubated with methionine at a concentration of 10, 100 or 1000 µg/ml in a non-standard microtitre assay for fluctuation, a higher number of revertants was observed in the absence of metabolic activation at the highest dose tested, but not in the presence of metabolic activation (Gatehouse, 1981). The authors suggested that excess methionine has an inhibitory effect on auxotrophs that require tryptophan, including this strain of *E. coli*, allowing spontaneous revertants to dominate the culture (Gatehouse, 1981). When *E. coli* strains WP2 and WP67 *uvr*A*pol*A, and CM871 *uvr*A*rec*A*lex*A were incubated with methionine at concentrations of up to 2500 µg/ml, slight increases in the number of revertants were reported in the presence of metabolic activation. The authors, however, were of the opinion that these results were an artefact of enhanced bacterial growth caused by the presence of a high concentration of methionine and the metabolic activation system, S9, and, therefore, concluded that the results of the test were negative (Green, 1981). In a similar study, using the same strains of *E. coli* (WP2, WP67 *uvr*A*pol*A and CM871 *uvr*A*rec*A*lex*A) D,L-methionine gave negative results with and without metabolic activation at concentrations of up to 1000 µg/ml (Tweats, 1981).

No evidence of mutagenicity was reported when various strains of *E. coli* were incubated with valine [FL-no: 17.023], L-histidine [FL-no: 17.008] or L-tyrosine [FL-no: 17.022] at concentrations of up to 5000 µg/plate without metabolic activation (Fluck et al., 1976; Martinez et al., 2000).

In a Rec assay using *Bacillus subtilis* H17 and M45, glycine [FL-no: 17.034], D,L-isoleucine [FL-no: 17.010], D,L-methionine [FL-no: 17.014], D,L-valine [FL-no: 17.023] and D,L-alanine [FL-no: 17.024] consistently gave negative results when incubated at concentrations up to 10000 µg/ml with and without metabolic activation (Kada, 1981; Kuroda et al., 1984a).

In a test designed to investigate potential induction of aneuploidy in *Saccharomyces cerevisiae* strain D6, D,L-methionine [FL-no: 17.014] at a concentration of 50 µg/ml was considered to produce a false positive response (Parry & Sharp, 1981). The authors concluded that the effect was caused by selective stimulation of growth of monosomic cells in a methionine-rich environment, at the expense of the original diploid cells, owing to loss of the wild-type allele of the *Met*13 gene in the monosomic cells. Although methionine (dissolved in dimethylsulfoxide) at concentrations of up to and including 750 µg/ml caused a doubling of the number of revertants compared with control values in a test evaluating mitotic gene conversion in *S. cerevisiae* strain JD1; the difference was not statistically significant (Sharp & Parry, 1981). Additionally, when distilled water was used as the solvent, the numbers of revertants observed with methionine at concentrations of up to and including 750 µg/ml were comparable to those in the controls. Overall, the authors concluded that methionine does not induce mitotic aneuploidy or gene conversion in the yeast strains tested (Parry & Sharp, 1981; Sharp & Parry, 1981). In *S. cerevisiae* strains D7 and D4, D,L-methionine at concentrations up to 1600 µg/ml and 333 µg/plate, respectively, tested negative for mitotic gene conversion with and without metabolic activation (Jagannath et al., 1981; Zimmermann & Scheel, 1981).

In an assay for forward mutation in mouse lymphoma L5178Y *Tk*+/- cells, D,L-methionine [FL-no: 17.014] and L-tyrosine [FL-no: 17.022] did not induce mutations at concentrations up to 3000 µg/ml and 271.8 µg/ml, respectively (Jotz & Mitchell, 1981; Garberg et al., 1988).

Fifteen amino acids [L-cysteine, FL-no: 17.033; glycine, FL-no: 17.034; L-isoleucine, FL-no: 17.010; leucine, FL-no: 17.012; L-methionine, FL-no: 17.014; L-proline, FL-no: 17.019; L-valine, FL-no: 17.023; L-phenylalanine, FL-no: 17.018; L-aspartic acid, FL-no: 17.005; L-glutamine, FL-no: 17.007; L-histidine, FL-no: 17.008; L-tyrosine, FL-no: 17.022; L-alanine,

FL-no: 17.024; L-arginine, FL-no: 17.003; L-lysine, FL-no: 17.026] at concentrations of 10, 50 and 100 µg/ml produced slight increases in sister chromatid exchanges (SCEs) in human lymphocytes. The elevated frequencies of SCE were similar at all three doses and were considered to be metabolic rather than genotoxic responses (Xing & Na, 1996). Furthermore, D,L-methionine (100–5000 µg/ml) and taurine [FL-no: 16.056] (125 µg/ml) showed no evidence of SCE in Chinese hamster ovary cells, with or without metabolic activation (Evans & Mitchell, 1981; Natarajan & van Kesteren-van Leeuwen, 1981; Perry & Thomson, 1981; Cozzi et al., 1995). Also, no SCEs were observed when Chinese hamster V79 cells were incubated with L-cysteine [FL-no: 17.033] (12.1–121 µg/ml) (Speit et al., 1980).

L-Cysteine [FL-no: 17.033], D,L-methionine [FL-no: 17.014], L-glutamine [FL-no: 17.007], L-tyrosine [FL-no: 17.022] and taurine [FL-no: 16.056] produced uniformly negative results in assays for chromosomal aberration when incubated at concentrations of up to 5000 µg/ml in Chinese hamster ovary cells (Natarajan & van Kesteren-van Leeuwen, 1981; Stich et al., 1981a; Cozzi et al., 1995; Tavares et al., 1998). Similarly, in Chinese hamster lung V79 fibroblasts incubated with methionine at concentrations of 45 to 1494 µg/ml, no increase in the frequency of chromosome aberrations was reported (Swenberg et al., 1976). In a study evaluating the potential induction of chromosomal aberrations in rat hepatocytes treated with methionine at doses of up to 200 µg/ml, results were reported to be inconclusive (Dean, 1981).

No unscheduled DNA synthesis (UDS) was observed when human fibroblast cells were incubated with D,L-methionine [FL-no: 17.014] at a concentration of up to 1000 µg/ml with metabolic activation (Agrelo & Amos, 1981). HeLa S3 cells incubated with D,L-methionine at a concentrations of up to 100 µg/ml also showed no evidence of UDS (Martin & McDermid, 1981). Furthermore, no UDS was observed in WI-38 human fibroblasts incubated with D,L-methionine at concentrations of 63 to 1000 µg/ml in the absence of metabolic activation. However, in the presence of metabolic activation, a weak UDS response was observed, which appeared to be dose-related at concentrations of 125 to 2000 µg/ml (Robinson & Mitchell, 1981).

In vivo

Three male and three female Wistar rats were given glutamine [FL-no: 17.007] as a single dose at 600 mg/kg bw by gavage. Preparations of metaphase cells were obtained from samples of bone marrow. When compared with untreated controls, rats receiving glutamine did not exhibit an increase in the number of chromosomal aberrations (Tavares et al., 1998).

Male CBA/J mice were given methionine [FL-no: 17.014] at a dose of 0, 1, 10, 100 or 1000 mg/kg bw by intraperitoneal injection. Partial hepatectomy was performed on half of the animals, which were then given up to 54 h to allow for liver regeneration. At termination, the mice were sacrificed and bone marrow and hepatocytes were harvested for analysis. It was reported that, overall, methionine did not induce SCE in the bone marrow of intact or partially hepatectomised mice (Paika et al., 1981).

In a modified assay for micronucleus formation in bone marrow, $B6C3F_1$ mice were injected intraperitoneally with methionine at a dose of 35 mg/kg bw at 0 and 24 h. Samples of bone marrow were harvested at 48, 72 and 96 h, and analysed for induction of micronuclei. Methionine was reported to cause increased formation of micronuclei in samples harvested at 72 h only. However, in a subsequent test conducted under the same conditions (samples of bone marrow obtained only at 48 and 72 h), methionine at a dose of 3.7, 17.5 or 35 mg/kg bw

did not increase the formation of micronuclei (Salamone et al., 1981). In another test for induction of micronuclei, CD-1 mice were given methionine at a dose of 0, 250, 500 or 1000 mg/kg bw as two intraperitoneal injections separated by an interval of 24 h. The mice were sacrificed 6 h after the last injection, and the bone marrow was harvested for analysis. There was no increase in the frequency of micronucleated polychromatic erythrocytes (Tsuchimoto & Matter, 1981).

Conclusion on genotoxicity

The amino acids tested were found to be non-genotoxic *in vitro* and *in vivo* in a variety of test systems.

For a summary of *in vitro/in vivo* genotoxicity data considered by the JECFA, see Table 2.1.

3.2. Genotoxicity Studies - Text Taken from EFSA (EFSA, 2008d)

In vivo

Data from *in vitro* tests are available for four candidate substances [FL-no: 17.006, 17.020, 17.021 and 17.031] and 16 supporting substances [FL-no: 17.003, 17.005, 17.007, 17.008, 17.010, 17.012, 17.014, 17.018, 17.019, 17.022, 17.023, 17.024, 17.026, 17.033, 17.034 and 16.056]. Data from *in vivo* tests are only available for two supporting substances [FL-no: 17.007 and 17.014].

Three candidates [FL-no: 17.006, 17.021 and 17.031] and 13 supporting substances [FL-no: 17.003, 17.005, 17.007, 17.008, 17.010, 17.012, 17.014, 17.017, 17.019, 17.022, 17.023, 17.024 and 17.034] tested for bacterial gene mutations gave negative results.

A weak increase in mutant frequency (1.5 fold above the control value) was observed with the supporting substance L-phenylalanine [FL-no: 17.018] in the absence of metabolic activation system in *E. coli uvrB* cells. The effect was suppressed by equimolar concentrations of tyrosine and phenylalanine, and was not confirmed by using the excision repair proficient wild type *E. coli*, or other DNA-repair deficient strains such as *uvrB umuC, uvrB lexA, uvrB recA* (Sargentini & Smith, 1986) or *uvrB S. typhimurium* cells (De Veer et al., 1994). The Panel considered that the study had little relevance as the weak increase probably was related to an indirect effect, with no predictive validity for genotoxicity in mammals.

Slightly increased incidence of SCE in human peripheral blood lymphocytes were reported for one candidate substance [FL-no: 17.020] and 13 supporting substances [FL-no: 17.003, 17.005, 17.007, 17.008, 17.010, 17.012, 17.014, 17.017, 17.019, 17.022, 17.023, 17.024 and 17.033], tested at 10, 50 and 100 microgram/ml (in excess of the amount present in the tissue culture media) in the absence of any metabolic system (Zhang & Yang, 1992; Xing & Na, 1996). Cytotoxicity was not evaluated during the studies and the effect was not dose-dependent. The authors hypothesised that addition of exogenous amino acids might result in an imbalance among the amino acids in the medium and cause metabolic disturbances in the cells (Zhang & Yang, 1992; Xing & Na, 1996). As an overall evaluation, the Panel considered the results to be inconclusive.

In addition, for the three candidate substances [FL-no: 17.006, 17.021 and 17.031] and the ten supporting substances [FL-no: 17.007, 17.010, 17.014, 17.019, 17.022, 17.023, 17.024, 17.033, 17.034 and 17.056] negative results were reported in a number of other *in vitro* test systems (Rec assay, chromosomal aberration test, SCE and mammalian cell gene mutation assay, i.e. mouse lymphoma tests) both in the absence and in the presence of metabolic

activation system in a wide range of concentrations, including those approaching or causing cytotoxicity.

The available *in vivo* studies on supporting substances [FL-no: 17.007 and 17.014] give no indication of a genotoxic potential *in vivo.*

In most cases the isomer tested was not specified, or tests have been carried out only with the L-isomer.

Conclusion on genotoxicity

For the substances in this group the available data and consideration on the chemical structure do not give rise to safety concern with respect to genotoxicity.

For a summary of *in vitro/in vivo* genotoxicity data considered by EFSA, see Table 2.2.

3.3. EFSA Considerations

For the substances in this group of 19 JECFA evaluated amino acids and related substances, the available data do not give rise to safety concern with respect to genotoxicity.

4. Application of the Procedure

4.1. Application of the Procedure to some Amino Acids and Related Substances by JECFA (JECFA, 2005c):

The JECFA was of the opinion that the use of the Procedure for the Safety Evaluation of Flavouring Agents was inappropriate for eleven members of the group of amino acids and related substances, namely, the nine L-form α-amino acids L-cysteine, L-leucine, L-phenylalanine, L-aspartic acid, L-glutamine, L-histidine, L-thyrosine, L-arginine, and L-lysine ([FL-no: 17.033, 17.012, 17.017, 17.005, 17.007, 17.008, 17.022, 17.003 and 17.026]), the one α-amino acids glycine ([FL-no: 17.034]) and the one α-imino acid L-proline ([FL-no: 17.019]). These substances are macronutrients and normal components of protein and, as such, human exposure through food is orders of magnitude higher than the anticipated level of exposure from use as flavouring substances.

The JECFA also noted that amino acids may react with other food constituents upon heating. The mixtures thus formed are commonly referred to as "process flavours". The safety of process flavours has not been reviewed during this evaluation. The present evaluation is therefore on the basis that these flavouring substances are present in an unchanged form at the point of consumption.

The JECFA considered that for the remaining eight members of the group, the evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents. Although the D-form of the α-amino acids and the other three compounds are not found in protein, they are natural components of food. For these eight members of the group, the evaluation has been conducted only in relation to their use as flavouring substances leading to the current estimated intakes.

According to the JECFA seven of the substances, evaluated through the Procedure, belong to structural class I [FL-no: 16.056, 17.001, 17.010, 17.014, 17.017, 17.023 and 17.024] and one substance to structural class III [FL-no: 17.015] using the decision tree approach presented by Cramer et al. (1978).

The JECFA concluded the eight amino acids and related substances at step A3 in the JECFA Procedure – i.e. the substances are expected to be metabolised to innocuous products (step 2) and the intakes for all substances are below the thresholds for their structural classes I and III (step A3).

In conclusion the JECFA concluded that all eight substances evaluated through the Procedure to be of no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach.

The evaluations of the eight amino acids and related substances are summarised in Table 3.1: Summary of Safety Evaluation of Amino Acids and Related Substances (JECFA, 2005c).

4.2. Application of the Procedure to some Amino Acids From Chemical Group 34 by EFSA (EFSA, 2008d):

Nine candidate substances were evaluated in FGE.26Rev1. Three substances are classified into structural class I using the decision tree approach presented by Cramer et al. (1978). Six substances were not taken through the Procedure because the human exposure is orders of magnitude higher than the anticipated levels of exposure from the use of the flavouring substances. However, the Panel concluded that the substances were not of safety concern at their estimated levels of intake as flavouring substances.

The three substances, evaluated through the Procedure, were concluded at step A3 – i.e. the substances are expected to be metabolised to innocuous products (step 2) and the estimated daily intakes are below the threshold for the structural class I (step A3).

The Panel concluded that the three substances evaluated through the Procedure would not be of safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach.

The stepwise evaluations of the nine substances are summarised in Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA, 2008d).

4.3. EFSA Considerations

The Panel agrees with the evaluation as performed by the JECFA for the 19 substances in the group of amino acids and related substances.

The Panel noted that amino acids may react with other food constituents 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 on the basis that the present flavouring substances are in an unchanged form when they are consumed, thus in food that is not intended to be heated.

However, for five substances [FL-no: 16.056, 17.001, 17.003, 17.015 and 17.026] no European production figures were available for use as flavouring substances and consequently no European exposure estimates could be calculated. Accordingly, the safety in use in Europe could not be assessed using the Procedure for these five substances.

CONCLUSION

The Panel concluded that the 19 substances in the JECFA flavouring group of amino acids and related substances are structurally related to the nine amino acids from chemical group 34 evaluated by EFSA in the Flavouring Group Evaluation 26, Revision 1(FGE.26Rev1).

One further substance (L-glutamic acid, JECFA-no: 1420) was evaluated by the JECFA in this group. This substance is not in the Register and was therefore not dealt with in this consideration.

The Panel agrees with the evaluation as performed by the JECFA for the 19 substances considered in this FGE.

The Panel noted that amino acids may react with other food constituents 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 on the basis that the present flavouring substances in question are in an unchanged form when they are consumed, namely in food that is not intended to be heated.

For five substances [FL-no: 16.056, 17.001, 17.003, 17.015 and 17.026] the JECFA evaluation is only based on MSDI values derived from production figures from the USA. EU production figures are needed in order to finalise the evaluation of these substances.

For all substances use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the 19 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications:

Specifications including complete purity criteria and identity tests are available for all the 19 JECFA evaluated substances.

Thus the Panel concluded that the use of the Procedure was inappropriate for nine L-amino acids, glycine and one α-imino acid [FL-no: 17.003, 17.005, 17.007, 17.008, 17.012, 17.018, 17.019, 17.022, 17.026, 17.033 and 17.034] as the human exposures through food are in orders of magnitude higher than the anticipated levels of exposure from the use as flavouring substances. Therefore, these flavouring substances are not taken through the Procedure. However the Panel concluded that nine of the substances were not of safety concern at their estimated levels of intake as flavouring substances. For two substances [FL-no: 17.003 and 17.026] no European production figures were available and the evaluation could not be finalised for these.

For three of the eight JECFA evaluated amino acids and related substances (using the Procedure) [FL-no: 16.056, 17.001 and 17.015] the Panel has reservations (no European production volumes available, preventing evaluation using the Procedure). For the remaining five substances [FL-no: 17.010, 17.014, 17.017, 17.023 and 17.024] the Panel agrees with the JECFA conclusion "no safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY FOR JECFA EVALUATED SUBSTANCES IN THE PRESENT GROUP

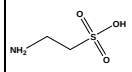
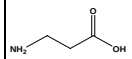
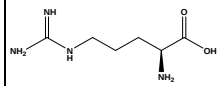
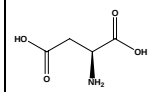
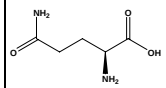
Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Amino Acids and Related Substances								
FL-no JECFA-no	**EU Register name**	**Structural formula**	**FEMA no CoE no CAS no**	**Phys. form Mol. formula Mol. weight**	**Solubility 1) Solubility in ethanol 2)**	**Boiling point, °C 3) Melting point, °C ID test Assay minimum**	**Refrac. Index 4) Spec. gravity 5)**	**EFSA comments**
16.056 1435	Taurine		3813 107-35-7	Solid $C_2H_7O_3NS$ 125.15	Soluble Soluble	 >300° NMR 98 %	n.a. n.a.	
17.001 1418	beta-Alanine		3252 107-95-9	Solid $C_3H_7O_2N$ 89.09	Soluble Slightly soluble	 202-207 NMR 97 %	n.a. n.a.	
17.003 1438	l-Arginine		3819 11890 74-79-3	Solid $C_6H_{14}O_2N_4$ 174.20	Soluble Slightly soluble	 222 MS 98 %	n.a. n.a.	According to JECFA: "Sp rotation = +15 to +17° (20°, 6N HCl)".
17.005 1429	Aspartic acid	 L-Aspartic acid shown	3656 10078 56-84-8	Solid $C_4H_7O_4N$ 133.10	Slightly soluble Insoluble	 270-271 MS 98 %	n.a. n.a.	Register name to be changed to L-Aspartic acid. According to JECFA: "Sp.rotation = +12.2 to +13.3° (25°, 5N HCl)".
17.007 1430	Glutamine	 L-Glutamine shown	3684 56-85-9	Solid $C_5H_{10}O_3N_2$ 146.15	Soluble Insoluble	 190-192 IR 98 %	n.a. n.a.	Register name to be changed to L-Glutamine. According to JECFA: "Sp.rotation = +15.5 to +16.5° (25°, 1N HCl)".

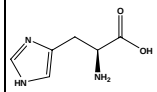
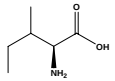
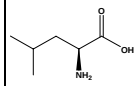
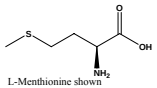
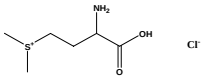
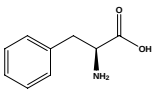
Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Amino Acids and Related Substances								
FL-no JECFA-no	**EU Register name**	**Structural formula**	**FEMA no CoE no CAS no**	**Phys. form Mol. formula Mol. weight**	**Solubility 1) Solubility in ethanol 2)**	**Boiling point, °C 3) Melting point, °C ID test Assay minimum**	**Refrac. Index 4) Spec. gravity 5)**	**EFSA comments**
17.008 1431	l-Histidine		3694 71-00-1	Solid $C_6H_9O_2N_3$ 155.16	Soluble Slightly soluble	 282 MS 98 %	n.a. n.a.	According to JECFA: "Sp.rotation = +37.5 to +39.5° (25°)".
17.010 1422	d,l-Isoleucine	 L-Isoleucine shown	3295 10127 443-79-8	Solid $C_6H_{13}O_2N$ 131.17	Soluble Insoluble	 290-292 IR 98 %	n.a. n.a.	
17.012 1423	l-Leucine		3297 10482 61-90-5	Solid $C_6H_{13}O_2N$ 131.17	Soluble Slightly soluble	 293-295 IR 98 %	n.a. n.a.	According to JECFA: "Sp. rotation = -9.5 to -11.5° (20°)".
17.014 1424	d,l-Methionine	 L-Menthionine shown	3301 569 59-51-8	Solid $C_5H_{11}O_2NS$ 149.21	Soluble Soluble	 281 MS 98 %	n.a. n.a.	
17.015 1427	S-Methylmethioninesulphonium chloride		3445 761 1115-84-0	Solid $C_6H_{14}O_2NS$ 199.70	Soluble Soluble	 139 NMR 98 %	n.a. n.a.	Register name to be changed to L-Methylmethioninesulphonium chloride.
17.017 1432	DL-Phenylalanine	 L-Phenylalanine shown	3726 10488 150-30-1	Solid $C_9H_{11}O_2N$ 165.19	Soluble Slightly soluble	 271-273 MS 98 %	n.a. n.a.	

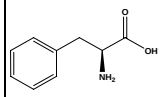
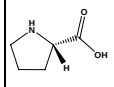
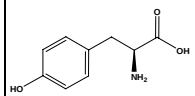
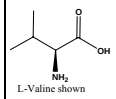
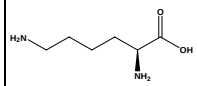
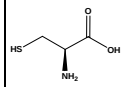
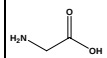
Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Amino Acids and Related Substances								
FL-no JECFA-no	**EU Register name**	**Structural formula**	**FEMA no CoE no CAS no**	**Phys. form Mol. formula Mol. weight**	**Solubility 1) Solubility in ethanol 2)**	**Boiling point, °C 3) Melting point, °C ID test Assay minimum**	**Refrac. Index 4) Spec. gravity 5)**	**EFSA comments**
17.018 1428	l-Phenylalanine		3585 10488 63-91-2	Solid $C_9H_{11}O_2N$ 162.19	Soluble Slightly soluble	270-275 MS 98 %	n.a. n.a.	According to JECFA: "Sp.rotation = -17.6 to -18.6° (20°)".
17.019 1425	l-Proline		3319 10490 147-85-3	Solid $C_5H_9O_2N$ 115.13	Soluble Soluble	220-222 NMR 98 %	n.a. n.a.	According to JECFA: "Sp.rotation = -80 to -82° (20°)".
17.022 1434	l-Thyrosine		3736 60-18-4	Solid $C_9H_{11}O_3N$ 181.19	Soluble Slightly soluble	342-344 IR 98 %	n.a. n.a.	Register name to be changed to L-Tyrosine. According to JECFA: "Sp.rotation = -4.5 to -5.5° (2°, 5N HCl)".
17.023 1426	DL-Valine	 L-Valine shown	3444 516-06-3	Solid $C_5H_{11}O_2N$ 117.15	Soluble Insoluble	295 MS 98 %	n.a. n.a.	
17.024 1437	DL-Alanine	 L-Alanine shown	3818 11729 302-72-7	Solid $C_3H_7O_2N$ 89.09	Soluble Slightly soluble	198 MS 98 %	n.a. n.a.	
17.026 1439	l-Lysine		3847 11947 56-87-1	Solid $C_6H_{14}O_2N_2$ 146.19	Soluble Slightly soluble	215 MS 97 %	n.a. n.a.	According to JECFA: "Sp.rotation = +12.5 to +13.5° (23°, 6N HCl)".

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Amino Acids and Related Substances								
FL-no JECFA-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)**	**EFSA comments**
17.033 1419	l-Cysteine		3263 10464 52-90-4	Solid $C_3H_7O_2NS$ 121.16	Very soluble Soluble	 240 MS 98 %	n.a. n.a.	According to JECFA: "Sp.rotation = +6.9 to +8.5° (30°)".
17.034 1421	Glycine		3287 11771 56-40-6	Solid $C_2H_5O_2N$ 75.07	Soluble Slightly soluble	 245 IR 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.*

n.a. not applicable

TABLE 2: GENOTOXICITY DATA

Table 2.1: Genotoxicity Data (*in vitro/in vivo*) for 19 Amino Acids and Related Substances (JECFA, 2006a)

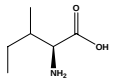
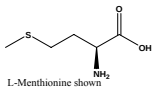
Table 2.1: Summary of Genotoxicity Data of 19 Amino Acids and Related Substances Evaluated by JECFA							
FL-no JECFA-no	**EU Register name JECFA name**	**Structural formula**	**End-point**	**Test system**	**Concentration**	**Results**	**Reference**
In vitro							
17.010 1422	d,l-Isoleucine	 L-Isoleucine shown	Recombination	*B. subtilis* H17 & M45	≤2000 µg/plate	Negative[g]	(Kuroda et al., 1984a)
			Recombination	*B. subtilis* H17 & M45	≤5000 µg/ml	Negative[g]	(Kuroda et al., 1984a)
			Sister chromatid exchange[s]	Human lymphocytes	47, 87 or 137 µg/ml[c]	Negative[d,e]	(Xing & Na, 1996)
17.014 1424	d,l-Methionine	 L-Menthionine shown	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	2-2000 µg/plate	Negative[b]	(Richold & Jones, 1981)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0.1-2000 µg/plate	Negative[c]	(Rowland & Severn, 1981)
			Reverse mutation	*S. typhimurium* TA98, TA100	≤1000000 µg/ml	Negative[e]	(Ichinotsubo et al., 1981b)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1537	5000 µg/plate	Negative[b]	(MacDonald, 1981)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1537	NR	Negative[b]	(Nagao & Takahashi, 1981)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	≤10000 µg/plate	Negative[b]	(Baker & Bonin, 1981)
			Reverse mutation	*S. typhimurium* TA92, TA98, TA100, TA1535, TA1537, TA1538	0.2, 2, 20, 200 or 2000 µg/plate	Negative[b,c]	(Brooks & Dean, 1981)

Table 2.1: Summary of Genotoxicity Data of 19 Amino Acids and Related Substances Evaluated by JECFA							
FL-no JECFA-no	**EU Register name JECFA name**	**Structural formula**	**End-point**	**Test system**	**Concentration**	**Results**	**Reference**
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	4, 20, 100, 500 or 2500 µg/plate	Negative[b]	(Trueman, 1981)
			Reverse mutation	*S. typhimurium* TA98, TA100	10, 20, 50, 100, 200 or 500 µg/plate	Negative[b]	(Venitt & Crofton-Sleigh, 1981)
			Mutation	*E. coli* WP2 & WP2 uvrA	≤500 µg/plate	Negative[b]	(Venitt & Crofton-Sleigh, 1981)
			Mutation	*E. coli* JC2921, JC5519, , JC7623, JC7689, JC8471, JC9239	≤1000000 µg/ml	Negative[f]	(Ichinotsubo et al., 1981a)
			Mutation	*E. coli* WP2 uvrA & WP2 uvrA(pKM101)	NR	Negative[c,h]	(Matsushima et al., 1981)
			Mutation	*E. coli* 343/113/uvrB & 343/113/uvrB/leu8	≤4000 µg/ml	Negative[b]	(Mohn et al., 1981)
			Mutation	*E. coli* WP2, WP67 uvrApolA & CM871 uvrArecAlexA	250, 500 or 1000 µg/ml	Negative[b]	(Tweats, 1981)
			Mutation	*E. coli* WP2, WP67 uvrApolA & CM871 uvrArecAlexA	2500 µg/ml	Negative[b]	(Green, 1981)
			Mutation	*E. coli* P3478	6000 µg/plate	Negative[e]	(Fluck et al., 1976)
			Mutation	*E. coli* WP2 uvrA	10-1000 µg/ml	Positive[e,f,i]	(Gatehouse, 1981)
			Mutation	*E. coli* WP2 uvrA	10-1000 µg/ml	Negative[f,i]	(Gatehouse, 1981)
			Recombination	*B. subtilis* H17rec+ & M45rec-	20 µl/disk	Negative[j]	(Kada, 1981)
			Mitotic gene conversion	*S. cerevisiae* D4	0.3-333 µg/plate	Negative[b]	(Jagannath et al., 1981)

Table 2.1: Summary of Genotoxicity Data of 19 Amino Acids and Related Substances Evaluated by JECFA							
FL-no JECFA-no	**EU Register name JECFA name**	**Structural formula**	**End-point**	**Test system**	**Concentration**	**Results**	**Reference**
			Aneuploidy	*S. cerevisiae* D6	50 µg/ml	Negative[b,k]	(Parry & Sharp, 1981)
			Mitotic gene conversion	*S. cerevisiae* D7	1600 µg/ml	Negative[f]	(Zimmermann & Scheel, 1981)
			Mitotic gene conversion	*S. cerevisiae* JD1	≤750 µg/ml	Negative[b]	(Sharp & Parry, 1981)
			Forward mutation	Mouse lymphoma L5178Y cells	46.9-3000 µg/ml	Negative[b]	(Jotz & Mitchell, 1981)
			Forward mutation	Mouse lymphoma L5178Y Tk+/-	0.0005-0.015 mol/l (74.6-2238 µg/ml)[m]	Negative[f,i]	(Garberg et al., 1988)
			Sister chromatid exchange	Human lymphocytes	21, 61 or 111 µg/ml[c]	Negative[d,e]	(Xing & Na, 1996)
			Sister chromatid exchange	Chinese hamster ovary cells	0.1, 1, 10 or 100 µg/ml	Negative[b]	(Perry & Thomson, 1981)
			Sister chromatid exchange	Chinese hamster ovary cells	1670, 3300 or 5000 µg/ml	Negative[g]	(Natarajan & van Kesteren-van Leeuwen, 1981)
			Sister chromatid exchange	Chinese hamster ovary cells	21.88-350 µg/ml	Negative[b]	(Evans & Mitchell, 1981)
			Chromosomal aberrations	Chinese hamster ovary cells	1670, 3300 or 5000 µg/ml	Negative[f]	(Natarajan & van Kesteren-van Leeuwen, 1981)
			Chromosomal aberration	Rat hepatocytes	50, 100 or 200 µg/ml	Inconclusive	(Dean, 1981)
			Chromosomal aberration	Chinese hamster lung fibroblast V79 cells	0.3, 1, 3, or 110 mmol/l (45-1494 µg/ml)[m]	Negative[b]	(Swenberg et al., 1976)
			Unscheduled DNA Synthesis	Human fibroblast cells	≤1000 µg/ml	Negative[f]	(Agrelo & Amos, 1981)

Table 2.1: Summary of Genotoxicity Data of 19 Amino Acids and Related Substances Evaluated by JECFA

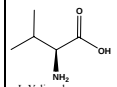
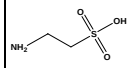
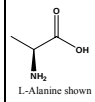
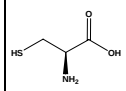
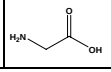
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			Unscheduled DNA synthesis	Human WI-38 fibroblasts	63–1000 µg/ml	Negative[e]	(Robinson & Mitchell, 1981)
			Unscheduled DNA synthesis[y]	Human WI-38 fibroblasts	125–2000 µg/ml	Positive[f]	(Robinson & Mitchell, 1981)
			Unscheduled DNA synthesis	HeLa S3 cells	0.1–100 µg/ml	Negative[b]	(Martin & McDermid, 1981)
17.023 1426	DL-Valine	 L-Valine shown	Mutation	*E. coli* P3478	500 µg/plate	Negative[e]	(Fluck et al., 1976)
			Recombination	*B. subtilis* H17 & M45	≤2000 µg/plate	Negative[g]	(Kuroda et al., 1984a)
			Recombination	*B. subtilis* H17 & M45	≤10000 µg/ml	Negative[g]	(Kuroda et al., 1984a)
			Sister chromatid exchange[t]	Human lymphocytes	25, 65 or 115 µg/ml[c]	Negative[d,e]	(Xing & Na, 1996)
16.056 1435	Taurine		Sister chromatid exchange	Chinese hamster ovary cells	10^{-3} mol/l (125 µg/ml)[p]	Negative[b]	(Cozzi et al., 1995)
			Chromosomal aberrations	Chinese hamster ovary cells	10^{-3} mol/l (125 µg/ml)[o]	Negative[b]	(Cozzi et al., 1995)
17.024 1437	DL-Alanine	 L-Alanine shown	Recombination	*B. subtilis* H17 & M45	≤2000 µg/plate	Negative[g]	(Kuroda et al., 1984a)
			Recombination	*B. subtilis* H17 & M45	≤10000 µg/ml	Negative[g]	(Kuroda et al., 1984a)
			Sister chromatid exchange[u]	Human lymphocytes	10, 50 or 100 µg/ml[c]	Negative[d,e]	(Xing & Na, 1996)
17.033 1419	l-Cysteine		Sister chromatid exchange	Chinese hamster V79 cells	10^{-4}–10^{-3} mol/l (12.1–121 µg/ml)[a]	Negative[b]	(Speit et al., 1980)
			Chromosomal aberrations	Chinese hamster ovary cells	5 x 10^{-4} mol/l (61 µg/ml)[a]	Negative	(Stich et al., 1981a)
			Sister chromatid exchange	Human lymphocytes	47, 87 or 137 µg/ml[c]	Negative[d,e]	(Xing & Na, 1996)
17.034 1421	Glycine		Reverse mutation	*S. typhimurium* TA97, TA98, TA102	100, 500, 1000, 10000 µg/plate	Negative[f,g]	(Fujita et al., 1994)

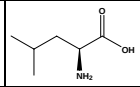
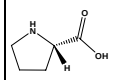
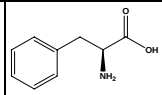
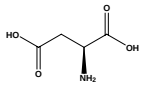
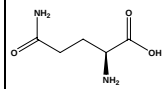
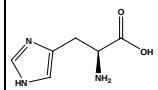
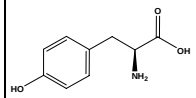
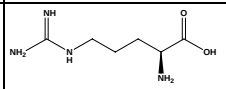
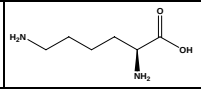
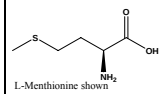
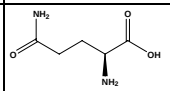
Table 2.1: Summary of Genotoxicity Data of 19 Amino Acids and Related Substances Evaluated by JECFA							
FL-no JECFA-no	**EU Register name JECFA name**	**Structural formula**	**End-point**	**Test system**	**Concentration**	**Results**	**Reference**
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	≤10000 µg/plate	Negative[f]	(Haworth et al., 1983)
			Recombination	*B. subtilis* H17 & M45	1660 µg/plate	Negative[b,g]	(Kuroda et al., 1984a)
			Sister chromatid exchange	Human lymphocytes	17, 57 or 107 µg/ml	Negative[d,e]	(Xing & Na, 1996)
17.012 1423	l-Leucine		Sister chromatid exchange	Human lymphocytes	47, 87 or 137 µg/ml[c]	Negative[d,e]	(Xing & Na, 1996)
17.019 1425	l-Proline		Reverse mutation	S. typhimurium TA1530, TA1531, TA1532, TA1964	0.5 mol/l (57550 µg/plate)n	Negative[b]	(Green & Savage, 1978)
			Sister chromatid exchange	Human lymphocytes	25, 65 or 115 µg/mlc	Negative[d,e]	(Xing & Na, 1996)
17.018 1428	l-Phenylalanine		Sister chromatid exchange	Human lymphocytes	21, 61 or 111 µg/mlc	Negative[d,e]	(Xing & Na, 1996)
17.005 1429	Aspartic acid	 L-Aspartic acid shown	Sister chromatid exchangev	Human lymphocytes	25, 65 or 115 mg/ml	Negative[d,e]	(Xing & Na, 1996)
17.007 1430	Glutamine	 L-Glutamine shown	Sister chromatid exchangew	Human lymphocytes	229, 269 or 319 µg/mlc	Negative[d,e]	(Xing & Na, 1996)
			Chromosomal aberrations	Chinese hamster ovary cells	292.2 µg/ml	Negative	(Tavares et al., 1998)
17.008 1431	l-Histidine		Mutation	E. coli WP2 uvrA/pKM101 oxyR+ & oxyR- (IC203)	5000 µg/plate	Negative[e]	(Martinez et al., 2000)
			Sister chromatid exchange	Human lymphocytes	21, 61 or 111 µg/ml[c]	Negative[d,e]	(Xing & Na, 1996)

Table 2.1: Summary of Genotoxicity Data of 19 Amino Acids and Related Substances Evaluated by JECFA							
FL-no JECFA-no	**EU Register name JECFA name**	**Structural formula**	**End-point**	**Test system**	**Concentration**	**Results**	**Reference**
17.022 1434	l-Thyrosine		Mutation	E. coli WP2 uvrA/pKM101 oxyR+ & oxyR- (IC203)	1000 µg/plate	Negative[e]	(Martinez et al., 2000)
			Forward mutation	Mouse L5178Y/Tk+/-	0.00005–0.0015 mol/l (9.06–271.8 µg/ml)[o]	Negative[b]	(Garberg et al., 1988)
			Sister chromatid exchange	Human lymphocytes	25, 65 or 115 µg/ml[c]	Negative[d,e]	(Xing & Na, 1996)
			Chromosomal aberrations	Chinese hamster ovary cells	10^{-3} mol/l (181.2 µg/ml)[o]	Negative	(Stich et al., 1981a)
17.003 1438	l-Arginine		Sister chromatid exchange	Human lymphocytes	156, 196 or 246 µg/ml	Negative[d,e]	(Xing & Na, 1996)
17.026 1439	l-Lysine		Sister chromatid exchange	Human lymphocytes	39, 79 or 129 µg/ml[c]	Negative[d,e]	(Xing & Na, 1996)
In vivo							
17.014 1424	d,l-Methionine	 L-Menthionine shown	Sister chromatid exchange	Mouse	1, 10, 100 or 1000 mg/kg bw	Negative[q]	(Paika et al., 1981)
			Micronucleus formation	Mouse	3.7, 17.5 or 35 mg/kg bw	Negative[q]	(Salamone et al., 1981)
			Micronucleus formation	Mouse	250, 500 or 1000 mg/kg bw	Negative[q]	(Tsuchimoto & Matter, 1981)
17.007 1430	Glutamine	 L-Glutamine shown	Chromosomal aberrationx	Rat	600 mg/kg bw	Negative[r]	(Tavares et al., 1998)

NR, not reported.

a) *Calculated using relative molecular mass of l-cysteine = 121.2.*

b) *With or without metabolic activation.*

c) *Cumulative concentrations comprised of background levels present in the medium and test compound added at concentrations of 10, 50 or 100 mg/ml.*

d) *A slight increase in sister chromatid exchanges were attributed to the resulting metabolic imbalance.*

e) *Without metabolic activation.*

f) *With metabolic activation.*

g) *Article in Japanese.*

h) *Pre-incubation method.*

i) *Microtitre fluctuation test.*

j) *Owing to the selective growth inhibitory effect of methionine on auxotrophs that require tryptophan (Gatehouse, 1981).*

k) *With and without the spore method of metabolic activation.*

l) *Owing to selective growth of cells (Parry & Sharp, 1981).*

m) *Calculated using relative molecular mass of dl-methionin =149.2.*

n) *Calculated using relative molecular mass of l-proline=115.1.*

o) *Calculated using relative molecular mass for l-tyrosine=181.2.*

p) *Calculated using relative molecular mass for taurine=125.1.*

q) *Administered by the intraperitoneal route.*

r) *Administered by gavage.*

s) *Tested on l-Isoleucine.*

t) *Tested on l-Valine.*

u) *Tested on l-Alanine.*

v) *Tested on l-Aspartic acid.*

w) *Tested on l-Glutamine.*

x) *Tested on l-Glutamine.*

y) *Non-standard UDS test. Validity is questionable (Comment by EFSA).*

TABLE 2.2: GENOTOXICITY (*IN VITRO*) EFSA / FGE.26REV1

Substances listed in brackets are JECFA evaluated substances

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.26Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Glycine [17.034])	Ames assay	*S. typhimurium* TA97; TA102	10,000 µg/plate	Negative (+ S9)	(Fujita et al., 1994)	Article in Japanese. Data extracted from tables. The validity of the study cannot be evaluated.
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	Up to 10,000 µg/plate (5 doses tested)	Negative (±S9)	(Haworth et al., 1983)	Valid well conducted non GLP-study.
	Mutagenesis assay (plate method)	*E. coli uvrB; uvrB umu C*; *uvrB LexA*	2 mM (150 µg/ml)	Negative (-S9)	(Sargentini & Smith, 1986)	Valid non GLP-study although conducted in the absence of metabolic activation system.
	Rec assay	*B. subtilis* H17; M45	1660 µg/plate	Negative (±S9)	(Kuroda et al., 1984a)	Article in Japanese. Data extracted from tables. The validity of the study cannot be evaluated.
	Sister chromatid exchange	Human lymphocytes	Up to 107 µg/ml (3 doses tested)	Positive (-S9)	(Xing & Na, 1996)	Cytotoxicity was not measured. The effect was not dose-dependent. The results are considered inconclusive.
	Sister chromatid exchange	Human peripheral blood lymphocytes	Up to 100 µg/ml (3 doses tested)[1]	Positive (-S9)	(Zhang & Yang, 1992)	Isomer not specified. Cytotoxicity was not measured. The effect was not dose-dependent. The results are considered inconclusive.
(DL-Alanine [17.024])	Mutagenesis assay (plate method)	*E. coli uvrB; uvrB umu C*; *uvrB LexA*	2 mM (L-alanine) (178 µg/ml)	Negative (-S9)	(Sargentini & Smith, 1986)	Valid non GLP-study, although conducted in the absence of metabolic activation system.
	Rec assay	*B. subtilis* H17; M45	2000 µg/plate 10,000 µg/ml	Negative (±S9)	(Kuroda et al., 1984a)	Article in Japanese. Data extracted from tables. Validity of the study cannot be evaluated.
	Sister chromatid exchange	Human lymphocytes	Up to 100 µg/ml (L-alanine)	Positive (-S9)	(Xing & Na, 1996)	Cytotoxicity was not measured. The effect was not dose-dependence. The results are considered inconclusive.

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.26Rev1						
Chemical Name [FL-no]	**Test System**	**Test Object**	**Concentration**	**Result**	**Reference**	**Comments**
	Sister chromatid exchange	Human peripheral blood lymphocytes	Up to 100 µg/ml (3 doses testesd)[1]	Positive (-S9)	(Zhang & Yang, 1992)	Isomer not specified. Cytotoxicity was not measured. The effect was not dose-dependent. The results are considered inconclusive.
(DL-Valine [17.023])	Mutagenesis assay (plate method)	*E. coli uvrB; uvrB umu C*; *uvrB LexA*	2 mM (235 µg/ml)[2]	Negative[1] (-S9)	(Sargentini & Smith, 1986)	Valid non GLP-study although conducted in the absence of metabolic activation system.
	Mutation assay	*E. coli* P3478	500 µg/plate	Negative (±S9)	(Fluck et al., 1976)	Isomer not specified. Valid non GLP-study.
	Rec assay	*B. subtilis* H17; M45	2000 µg/plate 10,000 µg/ml	Negative (±S9)	(Kuroda et al., 1984a)	Article in Japanese. Data extracted from tables. The validity of the study cannot be evaluated.
	Sister chromatid exchange	Human lymphocytes	Up to 115 µg/ml (L-valine) (3 doses tested)	Positive (-S9)	(Xing & Na, 1996)	Cytotoxicity was not measured. The effect was not dose-dependent. The results are considered inconclusive.
	Sister chromatid exchange	Human peripheral blood lymphocytes	Up to 100 µg/ml (3 doses tested)[1]	Positive (-S9)	(Zhang & Yang, 1992)	Isomer not specified. Cytotoxicity was not measured. The effect was not dose-dependent. The results are considered inconclusive.
(l-Leucine [17.012])	Mutagenesis assay (plate method)	*E. coli uvrB; uvrB umu C*; *uvrB LexA*	2 mM (L-Leucine) (234 µg/ml)	Negative (-S9)	(Sargentini & Smith, 1986)	Valid non GLP-study although conducted in the absence of metabolic activation system
	Sister chromatid exchange	Human lymphocytes	Up to 137 µg/ml (3 doses tested)	Positive (-S9)	(Xing & Na, 1996)	Cytotoxicity was not measured. The effect was not dose-dependent. The results are considered inconclusive.
(d,l-Isoleucine [17.010])	Mutagenesis assay (plate method)	*E. coli uvrB; uvrB umu C* ; *uvrB LexA*	2 mM (L-Isoleucine) (262 µg/ml)	Negative (-S9)	(Sargentini & Smith, 1986)	Valid non GLP-study although conducted in the absence of metabolic activation system.
	Rec assay	*B. subtilis* H17; M45	2000 µg/plate 10,000 µg/ml	Negative (±S9)	(Kuroda et al., 1984a)	Article in Japanese. Data extracted from tables. The validity of the study cannot be evaluated.

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.26Rev1						
Chemical Name [FL-no]	**Test System**	**Test Object**	**Concentration**	**Result**	**Reference**	**Comments**
	Sister chromatid exchange	Human lymphocytes	Up to 137 µg/ml (L-Isoleucine) (3 doses testesd)	Positive (-S9)	(Xing & Na, 1996)	Cytotoxicity was not measured. The effect was not dose-dependent. The results are considered inconclusive.
	Sister chromatid exchange	Human peripheral blood lymphocytes	Up to 100 µg/ml (3 doses testesd)[1]	Negative (-S9)	(Zhang & Yang, 1992)	Isomer not specified.
(l-Phenylalanine [17.018])	Mutagenesis assay (plate method)	*E. coli uvrB; uvrB umu C*; *uvrB LexA*	2 mM (L-Phenylalanine) (330 µg/ml)	Negative (-S9) Positive (-S9) *(uvrB only)*	(Sargentini & Smith, 1986)	Valid non GLP-study although conducted in the absence of metabolic activation system. The positive result correspond to 1.5-fold increase vs. control. However, the study is of little relevance as it has no predictive validity for genotoxicity.
	Sister chromatid exchange	Human lymphocytes	Up to 111 µg/ml (L-Phenylalanine) (3 doses tested)	Positive (-S9)	(Xing & Na, 1996)	Cytotoxicity was not measured. The effect was not dose-dependence. The results are considered inconclusive.
	Sister chromatid exchange	Human peripheral blood lymphocytes	Up to 100 µg/ml (3 doses tested)[1]	Positive (-S9)	(Zhang & Yang, 1992)	Isomer not specified.Cytotoxicity was not measured. The effect was not dose-dependent. The results are considered inconclusive.
(l-Tyrosine [17.022])	Mutagenesis assay (plate method)	*E. coli uvrB;uvrB umu C*; *uvrB LexA*	2 mM (L-Tyrosine) (362 µg/ml)	Negative (-S9)	(Sargentini & Smith, 1986)	Valid non GLP-study although conducted in the absence of metabolic activation system.
	Mutoxitest	*E. coli* WP2 *uvrA/pkM101* *oxy*R$^+$ and *oxy*R$^-$ (IC203)	1000 µg/plate (D,L-Tyrosine)	Negative (±S9)	(Martinez et al., 2000)	Well conducted non GLP study.
	Forward mutation assay	Mouse lymphoma cells L5178Y/*tk*+/-	Up to15 mM (272 µg/ml) (L-Tyrosine) (control + 4 doses tested)	Negative (±S9)	(Garberg et al., 1988)	Well conducted non GLP study.

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.26Rev1						
Chemical Name [FL-no]	**Test System**	**Test Object**	**Concentration**	**Result**	**Reference**	**Comments**
	Sister chromatid exchange	Human lymphocytes	Up to 115 µg/ml (L-Tyrosine) (3 doses tested)	Positive (-S9)	(Xing & Na, 1996)	Cytotoxicity was not measured. The effect was not dose-dependent. The results are considered inconclusive.
	Chromosomal aberration assay	Chinese hamster ovary cells	10^{-3} M (181 µg/ml)	Negative	(Stich et al., 1981a)	Review. The detailed description of experimental system is not reported. The validity of the study cannot be evaluated.
Threonine [17.021]	Ames assay (plate incorporation method)	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537	Up to 10,000 µg/plate (3 doses tested)	Negative (±S9)	(Ishidate et al., 1984)	Valid non GLP-study.
	Mutagenesis assay (plate method)	*E. coli uvrB; uvrB umuC ; uvrB LexA*	2 mM (L-Threonine) (238 µg/ml)	Negative (-S9)	(Sargentini & Smith, 1986)	Valid non GLP-study although conducted in the absence of metabolic activation system.
	Sister chromatid exchange	Human peripheral blood lymphocytes	Up to 100 µg/ml (3 doses tested)[1]	Negative (-S9)	(Zhang & Yang, 1992)	Isomer not specified.
	Chromosomal aberration assay	Chinese hamster fibroblast cells	2 mg/ml (2000 µg/ml)	Negative (-S9)	(Ishidate et al., 1984)	Valid non GLP-study, although conducted in the absence of metabolic activation system.
Serine [17.020]	Sister chromatid exchange	Human peripheral blood lymphocytes	Up to 100 µg/ml (3 doses tested)[1]	Positive (-S9)	(Zhang & Yang, 1992)	Isomer not specified. Cytotoxicity was not measured. The effect was not dose-dependent. The results are considered inconclusive.
(L-Aspartic Acid [17.005])	Mutagenesis assay (plate method)	*E. coli uvrB; uvrB umuC ; uvrB LexA*	2 mM (L-Aspartic acid) (266 µg/ml)	Negative (-S9)	(Sargentini & Smith, 1986)	Valid non GLP-study although conducted in the absence of metabolic activation system.
	Sister chromatid exchange	Human lymphocytes	Up to 115 µg/ml (L-Aspartic acid) (3 doses tested)	Positive (-S9)	(Xing & Na, 1996)	Cytotoxicity was not measured. The effect was not dose-dependent. The results are considered inconclusive.
(L-Glutamine [17.007])	Mutagenesis assay (plate method)	*E. coli uvrB ;uvrB umuC; uvrB LexA*	2 mM (L-Glutamine) (292 µg/ml)	Negative (-S9)	(Sargentini & Smith, 1986)	Valid non GLP-study although conducted in the absence of metabolic activation system.

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.26Rev1						
Chemical Name [FL-no]	**Test System**	**Test Object**	**Concentration**	**Result**	**Reference**	**Comments**
	Sister chromatid exchange	Human lymphocytes	Up to 319 µg/ml (L-Glutamine) (3 doses tested)	Positive (-S9)	(Xing & Na, 1996)	Cytotoxicity was not measured. The effect was not dose-dependent. The results are considered inconclusive.
	Chromosomal aberration assay	Chinese hamster ovary cells	292 µg/ml	Negative (-S9)	(Tavares et al., 1998)	Isomer not specified. Valid non GLP-study although conducted in the absence of metabolic activation system.
(l-Lysine [17.026])	Sister chromatid exchange	Human lymphocytes	Up to 129 µg/ml (3 doses tested)	Positive (-S9)	(Xing & Na, 1996)	Cytotoxicity was not measured. The effect was not dose-dependent. The results are considered inconclusive.
	Sister chromatid exchange	Human peripheral blood lymphocytes	Up to 100 µg/ml (3 doses tested)[1]	Negative (-S9)	(Zhang & Yang, 1992)	Isomer not specified.
l-Lysine Monochlorhydrate [17.031]	Mutagenesis assay (plate method)	*E. coli uvrB; uvrB umuC*; *uvrB LexA*	2 mM (L-isomer) (292 µg/ml)	Negative (-S9)	(Sargentini & Smith, 1986)	Valid non GLP-study although conducted in the absence of metabolic activation system.
(l-Arginine [17.003])	Mutagenesis assay (plate method)	*E. coli uvrB; uvrB umuC*; *uvrB LexA*	2 mM (L-Arginine) (348 µg/ml)	Negative (-S9)	(Sargentini & Smith, 1986)	Valid non GLP-study although conducted in the absence of metabolic activation system.
	Sister chromatid exchange	Human lymphocytes	Up to 246 µg/ml (L-Arginine) (3 doses tested)	Positive (-S9)	(Xing & Na, 1996)	Cytotoxicity was not measured. The effect was not dose-dependent. The results are considered inconclusive.
	Sister chromatid exchange	Human peripheral blood lymphocytes	Up to 100 µg/ml (3 doses tested)[1]	Negative (-S9)	(Zhang & Yang, 1992)	Isomer not specified is questionable.
(l-Proline [17.019])	Ames assay (plate incorporation method)	*S. typhimurium* TA1530; TA1531; TA1532; TA1964	0.5 M (28,000 µg/plate)	Negative (±S9)	(Green & Savage, 1978)	Limited quality. Reference compound of a large study, details are reported for positive compounds only.
	Mutagenesis assay (plate method)	*E. coli uvrB; uvrB umuC*; *uvrB LexA*	2 mM (L-Proline) (230 µg/ml)	Negative (-S9)	(Sargentini & Smith, 1986)	Valid non GLP-study although conducted in the absence of metabolic activation system.
	Sister chromatid exchange	Human lymphocytes	Up to 115 µg/ml (L-Proline) (3 doses tested)	Positive (-S9)	(Xing & Na, 1996)	Cytotoxicity was not measured. The effect was not dose-dependent. The results are considered inconclusive.

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.26Rev1						
Chemical Name [FL-no]	**Test System**	**Test Object**	**Concentration**	**Result**	**Reference**	**Comments**
(l-Histidine [17.008])	Mutagenesis assay (plate method)	*E. coli uvrB; uvrB umuC*; *uvrB LexA*	2 mM (L-Histidine) (310 µg/ml)	Negative (-S9)	(Sargentini & Smith, 1986)	Valid non GLP-study although conducted in the absence of metabolic activation system.
	Mutoxitest	*E. coli* WP2 *uvrA/pkM101* *oxy*R$^+$ and *oxy*R$^-$ (IC203)	5000 µg/plate (L-Histidine)	Negative (±S9)	(Martinez et al., 2000)	Well conducted non GLP study.
	Sister chromatid exchange	Human lymphocytes	Up to 111 µg/ml (L-Histidine) (3 doses tested)	Positive (-S9)	(Xing & Na, 1996)	Cytotoxicity was not measured. The effect was not dose-dependent. The results are considered inconclusive.
	Sister chromatid exchange	Human peripheral blood lymphocytes	Up to 100 µg/ml (3 doses tested)[1]	Positive (-S9)	(Zhang & Yang, 1992)	Isomer not specified. Cytotoxicity was not measured. The effect was not dose-dependent. The results are considered inconclusive.
(l-Cysteine [17.033])	Sister chromatid exchange	V79 Chinese hamster ovary cells	Up to 10^{-3} M (121 µg/ml) (2 doses tested)	Negative (±S9)	(Speit et al., 1980)	Isomer not specified. Cytotoxicity was not measured. Limited relevance.
	Chromosomal aberration assay	Chinese hamster ovary cells	5×10^{-4} M (61 µg/ml)	Negative	(Stich et al., 1981a)	Review. The detailed description of experimental system is not reported. The validity of the study cannot be evaluated.
(d,l-Methionine [17.014])	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Up to 2000 µg/plate (control + 4 doses tested)	Negative (±S9)	(Richold & Jones, 1981)	Isomer not specified. Well conducted non GLP study.
	Ames assay (modified plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Up to 2000 µg/plate (control + 6 doses tested)	Negative (±S9)	(Rowland & Severn, 1981)	Well conducted non GLP study, although the tested isomer was not specified.
	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 1 g/ml	Negative (±S9)	(Ichinotsubo et al., 1981a)	Report of limited quality: experimental details on test concentrations, isomer tested, quantitative response are not given. Positive and negative control not reported.

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.26Rev1						
Chemical Name [FL-no]	**Test System**	**Test Object**	**Concentration**	**Result**	**Reference**	**Comments**
	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1537	5000 µg/plate	Negative (±S9)	(MacDonald, 1981)	Well conducted non GLP study, although the isomer tested was not specified.
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1537	Not reported	Negative (±S9)	(Nagao & Takahashi, 1981)	Report of limited quality: positive and negative control not reported, experimental details on concentration and isomer tested not given.
	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	10,000 µg/plate	Negative (±S9)	(Baker & Bonin, 1981)	Positive and negative control not reported, experimental details on isomer tested not given.
	Ames assay (preincubation method)	*S. typhimurium* TA92; TA98; TA100; TA1535; TA1537; TA1538	Up to 2000 µg/plate (control + 5 doses tested)	Negative (±S9)	(Brooks & Dean, 1981)	Well conducted non GLP study, although the isomer tested was not specified.
	Ames assay (modified plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Up to 2500 µg/plate (control + 5 doses tested)	Negative (±S9)	(Trueman, 1981)	Well conducted non GLP study, although the isomer tested was not specified.
	Ames assay	*E. coli* WP2 *uvrA;* WP2 *uvrA* (pKM101)	Not reported	Negative (±S9)	(Matsushima et al., 1981)	Report of limited quality: positive and negative control not reported, experimental details on concentration and isomer tested not given.
	Mutation assay	*E. coli* JC2921; JC5519; JC7623; JC7689; JC8471; JC9238	Up to 1 g/ml	Negative (±S9)	(Ichinotsubo et al., 1981b)	Report of limited quality: experimental details on test concentrations, isomer tested, quantitative response are not given.
	Liquid suspension mutation assay	*E. coli* 343/113/*uvr*B; 343/113/*uvr*B/*leu*8	Up to 4000 µg/ml	Negative (±S9)	(Mohn et al., 1981)	Report of limited quality: experimental details on test concentrations, isomer tested, quantitative response are not given.
	Mutation assay	*E. coli* P3478	6-500 µg/plate	Negative	(Fluck et al.,	Well conducted non GLP-study.

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.26Rev1						
Chemical Name [FL-no]	**Test System**	**Test Object**	**Concentration**	**Result**	**Reference**	**Comments**
			(D,L-Methionine)	(±S9)	1976)	
	Mutation assay	*E. coli* WP2*;* WP2 *uvr*A*pol*A; CM871 *Uvr*A*rec* A*lex*A	Up to 2500 µg/ml (control + 3 doses tested)	Negative (±S9)	(Green, 1981)	Well conducted non GLP study, although the isomer tested was not specified.
	Differential killing test	*E. coli* WP2*;* WP2 *uvr*A*pol*A; CM871 *uvr*A*rec*A*lex*A	Up to 1000 µg/ml (control + 3 doses tested)	Negative (±S9)	(Tweats, 1981)	Positive and negative control not reported, experimental details on isomer tested not given.
	Mutagenesis assay (plate method)	*E. coli uvrB; uvrB umu C* ; *uvrA LexA*	2 mM (L-methionine) (30 µg/ml)	Negative (-S9)	(Sargentini & Smith, 1986)	Valid non GLP-study although conducted in the absence of metabolic activation system.
	Rec assay	*B. subtilis* H17*rec*$^{+}$; M45*rec*$^{-}$ [3]	20 µl/disk (26,000 µg/disk)	Negative (±S9)	(Kada, 1981)	Isomer not specified. Well conducted and reported although unconventional genotoxicity test.
	Mitotic aneuploidy assay	*S. cerevisiae* D6	50 µg/ml	Negative (±S9)	(Parry & Sharp, 1981)	Unconventional genotoxicity test, limited relevance. Isomer not specified.
	Mitotic gene conversion	*S. cerevisiae* JD1	750 µg/ml	Negative (±S9)	(Sharp & Parry, 1981)	Unconventional genotoxicity test, limited relevance. Isomer not specified
	Mitotic gene conversion	*S. cerevisiae* D4	333 µg/plate	Negative (±S9)	(Jagannath et al., 1981)	Unconventional genotoxicity test, limited relevance. Isomer not specified
	Mitotic gene conversion	*S. cerevisiae* D7	1600 µg/ml	Negative (±S9)	(Zimmermann & Scheel, 1981)	Unconventional genotoxicity test, limited relevance. Isomer not specified
	Forward mutation assay	Mouse lymphoma cells L5178Y/*tk*+/-	Up to 15 mM (2238 µg/ml) (control + 3 doses tested)	Negative	(Garberg et al., 1988)	Isomer not specified. Well conducted non GLP study.
	Forward mutation assay	Mouse lymphoma cells L5178Y	Up to 3000 µg/ml	Negative (±S9)	(Jotz & Mitchell, 1981)	Well conducted study, although the isomer tested was not specified.

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.26Rev1						
Chemical Name [FL-no]	**Test System**	**Test Object**	**Concentration**	**Result**	**Reference**	**Comments**
	Chromosomal aberration assay	Chinese hamster ovary cells	Up to 5000 µg/ml	Negative (±S9)	(Natarajan & van Kesteren-van Leeuwen, 1981)	Isomer not specified. Well conducted study.
	Chromosomal aberration assay	Rat liver epythelial-like cell line (RL_1)	200 µg/ml	Negative	(Dean, 1981)	Isomer not specified. Unconventional genotoxicity test, limited relevance.
	DNA damage (alkaline eluition assay)	Chinese hamster lung fibroblast V79 cells	Up to 10 mM (1494 µg/ml) (control + 9 doses tested)	Negative (±S9)	(Swenberg et al., 1976)	Isomer not specified. Well conducted study. Validity limited to screening purposes.
	Sister chromatid exchange	Human lymphocytes	Up to 111 µg/ml (L-methionine) (3 doses tested)	Positive (-S9)	(Xing & Na, 1996)	Cytotoxicity was not measured. The effect was not dose-dependence. The relevance of positivity is questionable.
	Sister chromatid exchange	Chinese hamster ovary cells	Up to 100 µg/ml (control + 4 doses tested)	Negative (±S9)	(Perry & Thomson, 1981)	Isomer not specified . Well conducted and reported study.
	Sister chromatid exchange	Chinese hamster ovary cells	Up to 5000 µg/ml	Negative (±S9)	(Natarajan & van Kesteren-van Leeuwen, 1981)	Isomer not specified. Well conducted study.
	Sister chromatid exchange	Chinese hamster ovary cells	350 µg/ml	Negative (±S9)	(Evans & Mitchell, 1981)	Isomer not specified. Well conducted study.
	Unscheduled DNA synthesis	Human fibroblast cells	Up to 1000 µg/ml	Negative (±S9)	(Agrelo & Amos, 1981)	Well conducted study, although the isomer tested was not specified. Validity limited to screening purposes.
	Unscheduled DNA synthesis	HeLa S3 cells	100 µg/ml	Negative (+S9)	(Martin & McDermid, 1981)	Isomer not specified. Sufficient experimental details reported. Validity limited to screening purposes.

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.26Rev1						
Chemical Name [FL-no]	**Test System**	**Test Object**	**Concentration**	**Result**	**Reference**	**Comments**
	Unscheduled DNA synthesis	WI-38 human fibroblast cells	1000-2000 µg/ml	Negative (±S9)	(Robinson & Mitchell, 1981)	Isomer not specified. Sufficient experimental details reported. Validity limited to screening purposes.
(Taurine [16.056])	Chromosomal aberration assay	Chinese hamster ovary cells	10^{-3} M (125 µg/ml)	Negative (-S9)	(Cozzi et al., 1995)	Well conducted study, although only in the absence of metabolic system.
	Sister chromatid exchange	Chinese hamster ovary cells	10^{-3} M (125 µg/ml)	Negative (-S9)	(Cozzi et al., 1995)	Well conducted study, although only in the absence of metabolic system.
Cystine [17.006]	Mutagenesis assay (plate method)	*E. coli uvrB; uvrB umu C*; *uvrB LexA*	2 mM (L-Cystine) (481 µg/ml)	Negative (-S9)	(Sargentini & Smith, 1986)	Valid non GLP-study although conducted in the absence of metabolic activation system.

1) *In excess of the amount present in the tissue culture media (which was not specified).*

2) *L-Valine was tested in the presence of 2 mM L-isoleucine.*

3) *With and without spore method.*

NR: Not reported

TABLE 2.3: GENOTOXICITY (*IN VIVO*) EFSA / FGE.26REV1

Substances listed in brackets are JECFA evaluated substances

Table 2.3: Summary of Genotoxicity Data (*in vivo*) EFSA / FGE.26Rev1							
Chemical Name [FL-no]	**Test System**	**Test Object**	**Route**	**Dose**	**Result**	**Reference**	**Comments**
(L-Glutamine [17.007]	*In vivo* Chromosomal aberration test	Rat	Oral gavage	600 mg/ kg bw	Negative	(Tavares et al., 1998)	Isomer not specified.
(DL-Methionine [17.014])	*In vivo* Sister chromatid exchange	Mouse CBA/J	Intraperitoneal injection	1000 mg/kg bw	Negative	(Paika et al., 1981)	Isomer not specified.
	In vivo Micronucleus test	Mouse B6C3F1	Intraperitoneal injection	35 mg/kg bw	Negative	(Salamone et al., 1981)	Isomer not specified.
	In vivo Micronucleus test	Mouse CD-1	Intraperitoneal injection	1000 mg/kg bw	Negative	(Tsuchimoto & Matter, 1981)	Isomer not specified.

TABLE 3: SUMMARY OF SAFETY EVALUATION TABLES

Table 3.1: Summary of Safety Evaluation of Amino Acids and Related Substances (JECFA, 2005c)

Table 3.1: Summary of Safety Evaluation of 19 JECFA Evaluated Substances (JECFA, 2005c)

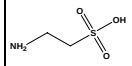
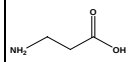
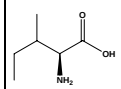
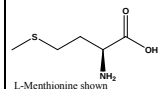
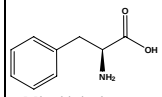
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (μg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [(4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
16.056 1435	Taurine		ND 217	Class I. A3: Intake below threshold.	4)	MSDI based on USA production figure.	MSDI based on USA production figure.
17.001 1418	beta-Alanine		ND 13	Class I. A3: Intake below threshold.	4)	MSDI based on USA production figure.	MSDI based on USA production figure.
17.010 1422	d,l-Isoleucine	 L-Isoleucine shown	5.2 22	Class I. A3: Intake below threshold.	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
17.014 1424	d,l-Methionine	 L-Menthionine shown	83 35	Class I. A3: Intake below threshold.	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
17.017 1432	DL-Phenylalanine	 L-Phenylalanine shown	1.9 0.7	Class I. A3: Intake below threshold.	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.

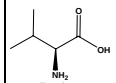
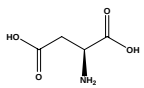
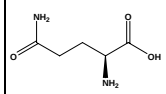
Table 3.1: Summary of Safety Evaluation of 19 JECFA Evaluated Substances (JECFA, 2005c)							
FL-no JECFA-no	**EU Register name**	**Structural formula**	**EU MSDI 1) US MSDI (μg/*capita*/day)**	**Class 2) Evaluation procedure path 3)**	**Outcome on the named compound [4) or 5)]**	**EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)**	**EFSA conclusion on the material of commerce**
17.023 1426	DL-Valine	 L-Valine shown	35 48	Class I. A3: Intake below threshold.	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
17.024 1437	DL-Alanine	 L-Alanine shown	115 1	Class I. A3: Intake below threshold.	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
17.005 1429	Aspartic acid	 L-Aspartic acid shown	68 1240	Class III. Not evaluated using the Procedure.	4)	6). No safety concern at estimated level of intake as flavouring substance.	Register name to be changed to L-Aspartic acid. 6). No safety concern at estimated level of intake as flavouring substance.
17.007 1430	Glutamine	 L-Glutamine shown	13 10	Class III. Not evaluated using the Procedure.	4)	6). No safety concern at estimated level of intake as flavouring substance.	Register name to be changed to L-Glutamine. 6). No safety concern at estimated level of intake as flavouring substance.

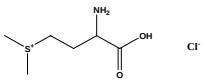
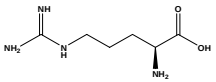
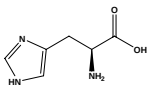
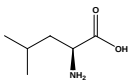
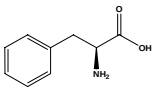
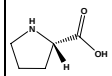
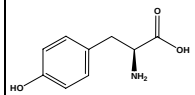
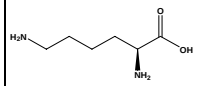
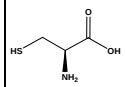
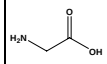
Table 3.1: Summary of Safety Evaluation of 19 JECFA Evaluated Substances (JECFA, 2005c)							
FL-no JECFA-no	**EU Register name**	**Structural formula**	**EU MSDI 1) US MSDI (µg/*capita*/day)**	**Class 2) Evaluation procedure path 3)**	**Outcome on the named compound [4) or 5)]**	**EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)**	**EFSA conclusion on the material of commerce**
17.015 1427	S-Methylmethioninesulphonium chloride		ND 75	Class III. A3: Intake below threshold.	4)	MSDI based on USA production figure.	Register name to be changed to L-Methylmethioninesulphonium chloride. MSDI based on USA production figure.
17.003 1438	l-Arginine		ND 57	Class III. Not evaluated using the Procedure.		6). MSDI based on USA production figures.	6). MSDI based on USA production figures.
17.008 1431	l-Histidine		9.5 259	Class III. Not evaluated using the Procedure.		6). No safety concern at estimated level of intake as flavouring substance.	6). No safety concern at estimated level of intake as flavouring substance.
17.012 1423	l-Leucine		12 24	Class III. Not evaluated using the Procedure.		6). No safety concern at estimated level of intake as flavouring substance.	6). No safety concern at estimated level of intake as flavouring substance.
17.018 1428	l-Phenylalanine		17 28	Class III. Not evaluated using the Procedure.		6). No safety concern at estimated level of intake as flavouring substance.	6). No safety concern at estimated level of intake as flavouring substance.

Table 3.1: Summary of Safety Evaluation of 19 JECFA Evaluated Substances (JECFA, 2005c)							
FL-no JECFA-no	**EU Register name**	**Structural formula**	**EU MSDI 1) US MSDI (µg/*capita*/day)**	**Class 2) Evaluation procedure path 3)**	**Outcome on the named compound [4) or 5)]**	**EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)**	**EFSA conclusion on the material of commerce**
17.019 1425	l-Proline		41 115	Class III. Not evaluated using the Procedure.		6). No safety concern at estimated level of intake as flavouring substance.	6). No safety concern at estimated level of intake as flavouring substance.
17.022 1434	l-Thyrosine		10 4	Class III. Not evaluated using the Procedure.		6). No safety concern at estimated level of intake as flavouring substance.	6). No safety concern at estimated level of intake as flavouring substance.
17.026 1439	l-Lysine		ND 57	Class III. Not evaluated using the Procedure.		6). MSDI based on USA production figures.	6). MSDI based on USA production figures.
17.033 1419	l-Cysteine		547 293	Class III. Not evaluated using the Procedure.		6). No safety concern at estimated level of intake as flavouring substance.	6). No safety concern at estimated level of intake as flavouring substance.
17.034 1421	Glycine		135 5	Class III Not evaluated using the Procedure.		6). No safety concern at estimated level of intake as flavouring substance.	6). No safety concern at estimated level of intake as flavouring substance.

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, Class II = 540, 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] *Not evaluated using the Procedure. The substance is a macronutrient which is a normal component of food protein and, as such, human exposure through food is orders of magnitude higher than the anticipated level of exposure from use as a flavouring substance.*

ND: not determined

TABLE 3.2: SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE (EFSA / FGE.26REV1)

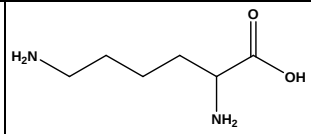
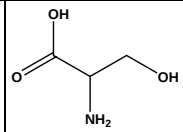
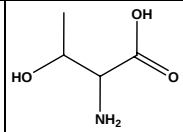
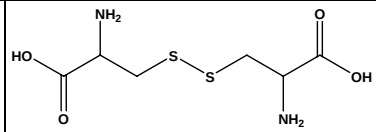
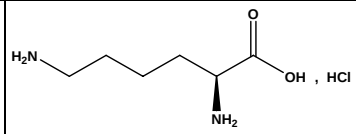
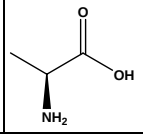
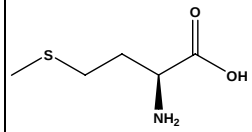
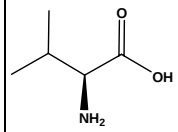
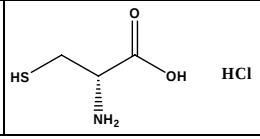
Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)							
FL-no	**EU Register name**	**Structural formula**	**MSDI 1) (µg/capita/day)**	**Class 2) Evaluation procedure path 3)**	**Outcome on the named compound [4) or 5]**	**Outcome on the material of commerce [6), 7), or 8)]**	**Evaluation remarks**
17.013	DL-Lysine		0.24	Class I A3: Intake below threshold	4)	6)	
17.020	Serine		0.61	Class I A3: Intake below threshold	4)	6)	
17.021	Threonine		0.12	Class I A3: Intake below threshold	4)	6)	
17.006	Cystine		2.4	Class I Not evaluated using the Procedure		6)	a)
17.031	l-Lysine monochlorhydrate		30	Class I Not evaluated using the Procedure		6)	b)
17.002	l-Alanine		450	Class I Not evaluated using the Procedure		7)	a)

Table 3.2: 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
17.027	l-Methionine		410	Class I Not evaluated using the Procedure		7)	a)
17.028	l-Valine		18	Class I Not evaluated using the Procedure		7)	a)
17.032	l-Cysteine hydrochloride		560	Class I Not evaluated using the Procedure		6)	b)

1) *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, Class II = 540, 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 1 (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 insufficient information on stereoisomerism.*

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

a) *The substance is a macronutrient which is a normal component of food protein and, as such, human exposure through food is orders of magnitude higher than the anticipated level of exposure from use as a flavouring substance.*

b) *The substance is the hydrochloride salt of a macronutrient which is a normal component of food proteins and, as such, human exposure through food is orders of magnitude higher than the anticipated level of exposure from use as a flavouring substance.*

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