

**Opinion of the Scientific Panel on Food Additives,
Flavourings, Processing Aids and Materials in Contact with Food (AFC)
on a request from the Commission related to**

N-Acetyl-L-methionine for use in foods for special medical purposes

adopted on 10 December 2003

SUMMARY

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food has been asked to evaluate N-acetyl-L-methionine as a source of L-methionine for use in foods for special medical purposes in children over one year and adults.

The Panel noted that N-acetyl-L-methionine is deacetylated in animals and humans to L-methionine. The bioavailability of methionine from N-acetyl-L-methionine is comparable to that from L-methionine used as such as similar mean plasma methionine concentrations were recorded in pigs and in humans after loading with equimolar quantities of each, as L-methionine and N-acetyl-L-methionine were equally utilised in healthy adult human males in terms of blood urea nitrogen levels and faecal and urinary nitrogen excretion, and as demonstrated in rat feeding studies measuring growth rate and protein efficiency ratio.

The Panel also noted that adequate data on bioavailability of L-methionine from N-acetyl-L-methionine in the presence of other N-acetyl amino acid derivatives are not available. When combining different N-acetylated amino acids as sources for the respective amino acids, the efficiency of their deacetylation may be affected. The bioavailability of amino acids from a product containing more than one acetylated nutrient source would have to be assessed as a part of the product development. Normally such information would be available as a part of a file supporting the intended use of the product.

The exposure to L-methionine from uses of N-acetyl-L-methionine in foods for special medical purposes will correspond to that of L-methionine if used as such, and therefore is unlikely to give rise to adverse health effects based on the present knowledge on methionine toxicity. The Panel concluded that N-acetyl-L-methionine as a source of L-methionine for use in foods for special medical purposes in children over one year of age and adults is not of concern from the safety point of view.

Key words

N-acetyl-L-methionine, NAM; CAS Registry Number: 65-82-7; foods for special medical purposes.

BACKGROUND

The Scientific Committee on Food (SCF) was asked in November 2001 to consider the safety of a number of substances as sources of nutrients for foods for particular nutritional uses. The evaluations could not be completed under the SCF mandate and continuation of this work now falls to the EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food.

N-acetyl-L-methionine is proposed by the petitioner as a source of the essential amino acid methionine for use in foods for special medical purposes (FSMPs) in children over one year of age and adults.

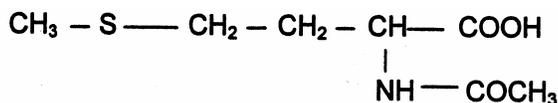
TERMS OF REFERENCE

The Commission asks the European Food Safety Authority (EFSA) to consider the safety and bioavailability of the nutrient source N-acetyl-L-methionine proposed for use in foods for particular nutritional uses.

ASSESSMENT

Chemistry

N-acetyl-L-methionine has a molecular weight of 191.25 of which 78% is L-methionine. Its melting point is 99-103°C. It has CAS Registry Number 65-82-7. Its chemical names are L-2-acetylamino-4-(methylthio)-butyric acid or 2-acetylamino-4-(methylthio)-butanoic acid. Its molecular formula is C₇H₁₃NO₃S and its structural formula is



It is in the form of white crystals or crystalline powder with weak characteristic odour.

Specifications

According to the petitioner, the purity of N-acetyl-L-methionine is not less than 99%. The loss on drying is below 0.3%. Possible chemical impurities include chloride limited to 200 mg/kg, sulphate limited to 500 mg/kg, ammonium limited to 200 mg/kg, heavy metals limited to 10 mg/kg, iron limited to 20 mg/kg, and other amino acids limited to 0.5%. The microbiological specification is as follows: aerobic plate count <1000/g, yeast and moulds <100/g, and specific organisms *Escherichia coli*, *Salmonella*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* not detectable in 1 g quantity. The specifications proposed by the petitioner conform to Food Chemical Codex IV (FCC IV, 1996).

Manufacturing process

According to the petitioner N-acetyl-L-methionine is produced by acetylation of L-methionine. The starting materials are L-methionine and acetic anhydride.

Methods of analysis in food

N-acetyl-L-methionine is analysed in amino-acid-based FSMPs by HPLC.

Reaction and fate in foods, stability

According to the petitioner N-acetyl-L-methionine is stable to chemical hydrolysis. It is more stable than L-methionine during Maillard reaction and Strecker degradation due to the protection of the amino group by acylation, but it is more sensitive to sulphoxide formation under some conditions of oxidation as demonstrated experimentally. This particular type of instability is, however, unlikely to be encountered in the processing of FSMPs in powdered or fluid forms.

Case of need and proposed uses

L-methionine is a permitted amino acid for use in foods for particular nutritional uses. According to the petitioner, replacement of L-methionine with N-acetyl-L-methionine improves the palatability of the product and enhances patient compliance with the diet without compromising the biological value of the formulation. Therefore N-acetyl-L-methionine is the preferred source of L-methionine in FSMPs for conditions such as inborn errors of metabolism, gastrointestinal conditions and food allergies.

N-acetyl-L-methionine is proposed as a source of methionine for use in FSMPs in children over one year of age and adults. Proposed uses by the petitioner for N-acetyl-L-methionine as a source of methionine are powdered, liquid and solid form FSMPs. According to the petitioner N-acetyl-L-methionine may be used as the only source of methionine in products based on amino acids as protein source or to supplement methionine in vegetable-derived proteins or hydrolysates.

Exposure

According to the petitioner the quantity of the N-acetyl-L-methionine added to products will generally be limited by the level required to meet requirements for methionine and levels of methionine used in existing FSMPs. The quantities will depend on product type i.e. a concentrated amino acid-based protein supplement such as those used in the dietary management of metabolic diseases will contain higher amounts than a nutritionally complete food.

As N-acetyl-L-methionine is proposed to replace L-methionine, the petitioner estimates the total daily intake of N-acetyl-L-methionine to be limited to a maximum of the total intake of methionine from different sources. According to the petitioner the typical daily intake of N-acetyl-L-methionine by patients with phenylketonuria will range from 0.86 g (providing 0.67 g of L-methionine) for a 1-2 years old child to 3 g (providing 2.34 g of L-methionine) for an adult in order to provide virtually all their daily intake of methionine.

For comparison, estimated mean daily requirements for methionine plus cysteine of healthy humans are 27 mg/kg bw for a child about 2 years old and 13 mg/kg bw for an adult. The population reference intake for adults is 17 mg/kg bw corresponding to 1 g/day for a 60 kg person (SCF, 1992).

Existing authorisations and evaluations

N-acetyl-L-methionine has been approved for use by the German authorities in dietetic foods for all age groups (BGBl, 1988).

In the USA, N-acetyl-L-methionine may be added to food (except infant foods and foods containing added nitrites/nitrates) as a source of L-methionine (Federal Register, 1978).

Biological and toxicological data

Bioavailability and metabolism

In vitro studies

N-acetyl-L-methionine is hydrolysed to L-methionine by acylase I (Baxter et al., 2002; Giardina, 1997), an enzyme found in several mammalian tissues, i.e. intestine, liver and kidney, which has a high affinity for sulphur and neutral amino acid derivatives (Giardina et al., 1997). The rat liver microsomal enzyme system has also been shown to deacetylate N-acetyl-methionine (Francis and Smith, 1975).

A study of *in vitro* deacetylation of N-acetyl-L-methionine, N-acetyl-histidine and N-acetyl-L-tryptophan (each substrate at the concentration of 4 mM) by extracts from mouse tissues showed that all activities were highest in the kidney and little in the blood, that most of N-acetyl-L-histidine was deacetylated by acylase I fraction and that deacetylation of N-acetyl-L-histidine in this fraction was inhibited by N-acetyl-L-methionine (Endo, 1980).

In vivo studies

A study of *in vivo* deacetylation of N-acetyl amino acids by kidney acylase I in mice demonstrated that the deacetylation of N-acetylhistidine was inhibited in the mouse kidney after the mixed intraperitoneal injection of N-acetylhistidine and N-acetyl-L-methionine (each 250 mg/kg bw) thus indicating higher affinity of the latter amino acid derivative for the acylase I (Endo, 1980).

In a series of experiments rats were dosed either with ¹⁴C-labelled N-acetyl-L-methionine or sodium acetate orally by gavage or intraperitoneally or with ³⁵S-labelled N-acetyl-L-methionine or L-methionine orally by gavage. With either route of dosing N-[1-¹⁴C]acetyl-L-methionine yield the same amount of ¹⁴CO₂ as sodium [1-¹⁴C]acetate over 24-hour period. The tissue distribution of ³⁵S from ³⁵S-labelled N-acetyl-L-methionine and L-methionine was similar 3, 24, and 168 hours after dosing. After 168 hours 30% of ³⁵S from both sources appeared in the urine and faeces, and the isotope was similarly distributed in the organic -S and inorganic -S fractions of urine. The experiments demonstrated that N-acetyl-L-methionine contributed as much acetate to the rats' acetate pool as an equimolar amount of sodium acetate, that both orally or intraperitoneally dosed N-acetyl-L-methionine were quantitatively cleaved to acetate and L-methionine, and that L-

methionine from N-acetyl-L-methionine was metabolically equivalent to free L-methionine (Rotruck and Bogds, 1975).

When equimolar amounts (2 mmol/kg bw) of L-methionine (298 mg/kg bw) and N-acetyl-L-methionine (382 mg/kg bw) were administered by gastrostomy tube to young pigs (n=4) the portal peak in methionine concentration was recorded later and was smaller in case of N-acetyl-L-methionine (90 minutes and concentration of 291 $\mu\text{mol}/100\text{ ml}$) compared to L-methionine (45 minutes and the concentration of 340 $\mu\text{mol}/100\text{ ml}$). Also the vena caval peak plasma level of methionine was recorded later and was slightly smaller in case of N-acetyl-L-methionine (90 minutes and concentration of 220 $\mu\text{mol}/100\text{ ml}$) compared to L-methionine (60 minutes and the concentration of 265 $\mu\text{mol}/100\text{ ml}$). N-acetyl-L-methionine could not be detected in either portal or vena caval plasma suggesting completed deacylation (hydrolysis) of N-acetyl-L-methionine in the intestinal lumen and/or mucosal cells (Daabees et al., 1984).

Several of the animal studies that conclude that the bioavailability of L-methionine and N-acetyl-L-methionine is similar are based on feeding studies measuring growth rate.

For N-acetyl-L-methionine the relative potency for weight gain in weanling male mice in a 14 day-growth assay has been calculated to be 89% of that obtained with L-methionine (Friedman and Gumbmann, 1987).

When N-acetyl-L-methionine was compared with L-methionine as a source of methionine in a 28 day-growth assay in weanling rats, N-acetyl-L-methionine produced an equivalent growth response and increase in protein efficiency ratios as L-methionine. Furthermore, an equivalent maximum growth response of rats fed L-methionine or N-acetyl-L-methionine was obtained when the total dietary sulphur amino acids comprised 0.36-0.41% of the diet (equivalent to intake of 0.36-0.41g/kg bw for a growing rat (Boggs et al., 1975).

Supplementation of a 10% protein basal diet with equimolar quantities of 0.224% L-methionine (equivalent to 0.224g/kg bw/day) or 0.289% N-acetyl-L-methionine (equivalent to 0.289g/kg bw/day) resulted in a significant increase in growth and protein efficiency ratio of weanling male rats fed a basal diet added each form of methionine for 28 days (Young Jenkins et al., 1978; *see also section Toxicological data*).

Human data

A 9-day metabolic balance study in healthy adult males (n=6) showed that L-methionine and N-acetyl-L-methionine were equally utilised in terms of blood urea nitrogen levels and faecal and urinary nitrogen excretion (Zezulka and Calloway, 1976).

A study in human adults (2 males and 3 females) comparing plasma and erythrocyte methionine levels during 4 hours after oral administration of equimolar quantities (0.0605 mmoles/kg bw) of L-methionine (9 mg/kg bw) and N-acetyl-L-methionine (12 mg/kg bw) dissolved in 300 ml distilled water containing 10% (w/v) sucrose found a slightly delayed absorption using N-acetyl-L-methionine reflected by a statistically significantly lower methionine plasma level at 15 minutes after administration but overall methionine plasma levels were similar. The AUC for methionine was similar after N-acetyl-L-methionine and after L-methionine administration. No evidence was

obtained that indicated presence of N-acetyl-L-methionine in the plasma or its excretion in urine after loading (Stegink et al., 1980).

A study in one year old infants (4 males and 4 females) using the same route of administration as in the previous study (Stegink et al., 1980) and the same equimolar quantities of L-methionine and N-acetyl-L-methionine dissolved in 160 ml distilled water containing 10% (w/v) sucrose demonstrated comparable overall methionine release from both compounds, but no difference in plasma methionine peak concentration or delay in absorption when using N-acetyl-L-methionine. The peak plasma methionine concentrations and AUC, for both compounds, in these infants was only half of that observed in normal adults (Stegink et al., 1982).

Toxicological data

The toxicological data submitted by the petitioner on N-acetyl-L-methionine were limited to two references (Young Jenkins et al., 1978; Rotruck and Boggs, 1977) in the original dossier.

In order to compare the efficacy of N-acetyl-L-methionine as a source of methionine for a food grade soy protein concentrate groups of 21-days old male weanling rats (N=8/group) were fed the following diets for 28 days: a basal diet containing 10% protein based on soy protein concentrate or the basal diet to which was added either 0.224% L-methionine (equivalent to 0.224g/kg bw) or 0.289% N-acetyl-L-methionine (equivalent to 0.289 g/kg bw providing 0.224 g/kg bw of L-methionine). Feed and water were provided *ad libitum*. Feed intake was recorded twice weekly and body weight once a week. At termination the blood samples were taken by heart puncture and the liver, testes, kidneys and spleen were weighed. Feed intake was comparable between the groups fed diets supplemented L-methionine or N-acetyl-L-methionine but was increased compared to the controls on basal diet, the difference reaching statistical significance for the L-methionine group only. Body weight gain and a protein efficiency ratio were not significantly different between groups fed L-methionine or N-acetyl-L-methionine supplemented diets, although these parameters were statistically significantly increased in both groups compared to the control group. All blood measures were comparable between the groups supplemented with L-methionine and N-acetyl-L-methionine (total protein, albumin, globulin, haemoglobin, blood urea nitrogen) and to those in the control group with an exception for total protein for which an increase in the N-acetyl-L-methionine group reached a statistical significance compared to the control group. The relative weights of liver, kidneys and testes were comparable in L-methionine and N-acetyl-L-methionine groups. In both groups, however, the relative weights of testes and kidneys were statistically significantly lower than in the control group. The relative spleen weight was statistically significantly increased in N-acetyl-L-methionine group compared to that in L-methionine and control groups. The authors concluded that based on growth and several biochemical parameters L-methionine and N-acetyl-L-methionine were equally well utilised by the rat (Young Jenkins et al., 1978).

In order to compare the effects of excessive dietary intakes of L-methionine and N-acetyl-L-methionine by measuring parameters which are known to be affected by excess methionine, male weanling rats (n=6/group) were fed different diets for 17 days: a basal diet based on an isolated soybean protein containing 10% protein and 0.15% methionine, or the basal diet to which was added supplemental L-methionine in concentrations ranging from 0.3% to 5% (equivalent to between 0.3g/kg bw/day and 5 g/kg bw/day) or equimolar levels of N-acetyl-L-methionine (equivalent to doses between 0.38 g/kg bw/day and 6.4 g/kg bw/day). Water and feed were supplied *ad libitum*. At termination spleens and blood were collected. After 14 days the body weight gain

and feed intake were highest in the groups fed diets containing 0.3% supplemental L-methionine or the equimolar level of supplemental N-acetyl-L-methionine. Higher levels of L-methionine or N-acetyl-L-methionine produced progressively less increase in body weight and feed intake and at 1.8% and higher concentrations body weight and feed intake were lower than in the control values. High levels of L-methionine (1.8%-3.0%) tended to more severely depress growth than did the equivalent amount of N-acetyl-L-methionine. Supplemental L-methionine at concentration of 1.2% and above or equivalent levels of N-acetyl-L-methionine caused comparable statistically significant increase in relative spleen weights, hypertrophy of the spleen and comparative statistically significant increase in spleen iron concentration when compared to controls from the basal diet group. Haematocrits were not statistically significantly different at all the levels of both compounds used. The authors concluded that the effects of excessive L-methionine or N-acetyl-L-methionine were a depression of growth and feed intake, spleen hypertrophy, and spleen iron accumulation in the growing rats and that N-acetyl-L-methionine at high levels of supplementation was less detrimental than L-methionine as evidenced by body weight gain (Rotruck and Boggs, 1977).

In the submission of additional information, a paper concerning the adequacy of acetyl-DL-methionine in supplementing DL-methionine deficient diets and on possible toxicity of acetyl-DL-methionine when administered in large doses to rats eating adequate amounts of food (Benedetti et al., 1968) was provided by the petitioner. The results generated with acetyl-DL-methionine were considered not directly applicable for evaluation of N-acetyl-L-methionine as a source of methionine.

Furthermore, the petitioner provided several data on toxicity of methionine. The information on toxicity of excess methionine is not directly applicable to the safety evaluation of N-acetyl-L-methionine as a source of L-methionine. Therefore the studies are not further discussed.

DISCUSSION

Mammalian tissues contain acylase-I, which is one of the enzymes enabling utilisation of amino acids from exogenous and endogenous acyl derivatives (including dipeptides and N-acetyl amino acids derived from protein hydrolysis). It was noted that deacetylation of N-acetylhistidine was inhibited in the mouse kidney after intraperitoneal injection of a mixture of N-acetylhistidine and N-acetyl-L-methionine. This suggests that the efficiency of deacetylation may be affected when specific mixtures of N-acetyl amino acids are ingested.

After loading with equimolar quantities of L-methionine and N-acetyl-L-methionine, *in vivo* studies in pigs and in humans demonstrated similar mean plasma methionine concentrations. However, plasma methionine concentrations peaked slightly later after administration of N-acetyl-L-methionine than after L-methionine. The observed delay and the fact that N-acetyl-L-methionine was not detected in portal or venal plasma indicate that N-acetyl-L-methionine is hydrolysed before the absorption in either the intestinal lumen and/or mucosal cells with the release of free methionine.

The delay in absorption does not affect the ability of N-acetyl-L-methionine to serve as a methionine source as demonstrated in animal feeding studies measuring growth rate and protein efficiency ratio. In these studies the bioavailability of L-methionine and N-acetyl-L-methionine was

similar. Also a metabolic balance study in humans supports equivalent utilisation of N-acetyl-L-methionine and L-methionine in terms of nitrogen retention.

Data on genotoxicity, reproductive and developmental toxicity of N-acetyl-L-methionine were not presented. Such toxicity studies are not needed in the light of the proposed levels of use and the hydrolysis of N-acetyl-L-methionine to physiological substrates (acetate and methionine).

Possible toxic effects of N-acetyl-L-methionine will be related to the toxicity of excessive L-methionine, which in animal manifests as depressed feed intake and body weight gain and tissue damage.

As N-acetyl-L-methionine is intended to replace L-methionine in FSMPs, the exposure to N-acetyl-L-methionine is estimated to correspond to the current intake of L-methionine from these foods.

CONCLUSIONS AND RECOMMENDATIONS

The Panel noted that the bioavailability of methionine from N-acetyl-L-methionine is comparable to that from L-methionine as similar mean plasma methionine concentrations were recorded in pigs and in humans after loading with equimolar quantities of each.

The Panel noted that adequate data on bioavailability of L-methionine from N-acetyl-L-methionine in the presence of other N-acetyl amino acid derivatives were not available. When combining different N-acetylated amino acids as sources for the respective amino acids, the efficiency of their deacetylation may be affected. The bioavailability of amino acids from a product containing more than one acetylated nutrient source would have to be assessed as a part of the product development. Normally such information would be available as a part of a file supporting the intended use of the product.

The exposure to L-methionine from uses of N-acetyl-L-methionine in foods for special medical purposes will correspond to that of L-methionine if used as such, and therefore is unlikely to give rise to adverse health effects based on the present knowledge on methionine toxicity.

The Panel concluded that N-acetyl-L-methionine as a source of L-methionine for use in foods for special medical purposes in children over one year of age and adults is not of concern from the safety point of view.

DOCUMENTATION PROVIDED TO EFSA

Original dossier on N-acetyl-L-methionine submitted via IDACE, March 2001.

Letter from the European Commission to the Chairman of the Scientific Committee on Food on "Evaluation of a number of substances added for specific nutritional purposes in foods for particular nutritional uses" dated 03/12/2002, Brussels. SCF/CS/ADD/NUT/50.

Additional submission related with the dossier: Additional information on: hydrolysis/bioavailability, toxicology.

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SCIENTIFIC PANEL (AFC) MEMBERS

R. Anton, S. Barlow, D. Boskou, L. Castle, R. Crebelli, W. Dekant, K.-H. Engel, S. Forsythe, W. Grunow, J.-C. Larsen, C. Leclercq, W. Mennes, M.-R. Milana, I. Rietjens, K. Svensson, P. Tobback, F. Toldrá.

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