Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to an application concerning the use of betaine as a novel food in the EU

(Request N° EFSA-Q-2004-090)

(adopted on 22 February 2005)

SUMMARY

This opinion refers to an application for the placing on the EU market of a novel food ingredient, betaine, intended for use in beverages, cereal products, confectionary and dairy products.

The Panel has addressed the main concerns raised by the Members States (MS) on the initial assessment report. In particular, the Panel has considered the available evidence on the safety of betaine from both animal and human studies. Based on these studies, a number of safety questions remain unresolved.

Animal studies showed treatment-related effects that were observed at all tested doses of betaine the biological or toxicological significance of which have not been satisfactorily clarified. Since the available studies do not allow the derivation of a no observed adverse effect level (NOAEL) for betaine and data on reproduction and developmental toxicity are lacking, an acceptable daily intake cannot be established.

The available data in human studies are insufficient to allow the Panel to rule out the safety concerns raised by the MS.

Therefore, it is concluded that the safety of betaine for the intended use as proposed by the applicant has not been established.

KEY WORDS

Betaine, trimethyl glycine, novel food ingredient, homocysteine, food safety.

BACKGROUND

In January 2003, Finnfeeds Finland Ltd. submitted a request under Article 4 of the Novel Food Regulation (EC) N° 258/97 to the Finnish authorities for placing on the market betaine as a food ingredient.

On 12 June 2003, the Finnish authorities forwarded to the Commission their initial assessment report of the product concerned carried out by the Novel Food Board (NFB) of the National Food Agency of Finland, which had reached the conclusion that the assumed daily intake of betaine would not be nutritionally disadvantageous for the consumer.

In accordance with Article 6(4) of the Novel Foods Regulation, the Commission forwarded the initial assessment report to Member States on 14 August 2004. Member States submitted their comments and/or presented reasoned objections within the 60 day period provided for in the authorisation procedure. In consequence, a Community Decision is now required and beforehand, it appears necessary to request a scientific opinion of the Authority as there might be an effect on public health (Article 11 of Regulation (EC) N° 258/97).

In its initial assessment report the NFB of Finland assumed that a daily intake of 20-30 g of the new ingredient in the foods concerned by the application would not be nutritionally disadvantageous for consumers. The NFB identified liver as the target organ of possible betaine toxicity. In this respect, the observed effects on animals were considered minor and at least largely reversible. However, it was noted also that the effects on the health of the consumer cannot be assessed with adequate reliability on the basis of the material presented by the applicant. The NFB hold the view that foods to which betaine has been added should not be marketed to pregnant women, breastfeeding mothers or infants.

The main concerns and suggestions raised by the Competent Authorities of other Member States are:

- There is insufficient information to assess the safety of this product for healthy individuals. In particular, concerns on the safety of betaine in the long term.
- Results of the animal studies do not prove satisfactorily that betaine is safe.
- Clinical studies are insufficient.
- There may be undesirable effects on glutathione and cholesterol metabolism.
- The product range proposed by the applicant is too broad.
- Vegetarians and those who are undernourished could run the risk of methionine deficiency by a supplemental intake of betaine.
- A potential interference with medical treatments of homocystinuria. Interactions of betaine with other nutrients (B_6, B_{12}, and folic acid) and choline derivates should be considered.
- Effects on the kinetics of methylation.
- The applicant did not submit any post-marketing surveillance plan.
- Risk management strategies should prevent intake by potentially vulnerable groups such as children and limit or prevent over consumption.

TERMS OF REFERENCE

In accordance with Article 29 (1) (a) of Regulation (EC) N° 178/2002, the European Commission requests the European Food Safety Authority to issue a scientific opinion on the use of betaine as a food ingredient in the context of Regulation (EC) N° 258/97.

The Authority is asked to specify whether the authorisation of betaine as a food ingredient is likely to have an effect on public health and, in particular, to focus on the elements of a scientific nature in the comments/objections raised by the Member States to the Initial Assessment Report.

ASSESSMENT

The application was considered to belong to category (e) of the Novel Foods Regulation, i.e. foods and food ingredients consisting of or isolated from plants or animals. In accordance with the Commission Recommendation 97/618/EC, the ingredient concerned by the application belongs to Class 1.1 "Pure chemicals or simple mixtures from non-GM sources; the source of the NF has a history of food use in the Community". Betaine is intended for use in beverages, cereal products, confectionary and dairy products. For this reason this Opinion will be an assessment of the safety data provided by the applicant to comply with the information required for novel foods of Class 1.1, i.e. information requirements I, II, III, IX, X, XI, XII and XIII as detailed in the following text.

i. Introduction to betaine

Betaine, also known as glycine betaine or trimethyl glycine, is present in most organisms. The principal physiologic role of betaine is as an osmolyte and methyl donor (Craig, 2004).

Several studies have suggested that betaine, along with other nutrients, helps to reduce levels of homocysteine (Borsook and Borsook, 1951a; Borsook and Borsook, 1951b; Graybiel and Patterson, 1951; Finkelstein and Martin, 2000; Matthews *et al.*, 2002; Schwab *et al.*, 2002; Ji and Kaplowitz, 2003; Olthof *et al.*, 2003; Steenge *et al.*, 2003). Elevated levels of plasma total homocysteine have been associated with a higher risk of cardiovascular disease (Finkelstein and Martin, 2000).

It has been reported that dietary betaine promotes the generation of hepatic S-adenosylmethionine and protects the human liver from ethanol-induced fatty infiltration (Barak *et al.*, 2003). Betaine may alter nutrient partitioning such that carcass protein deposition is enhanced at the expense of carcass fat and in part, visceral tissue (Fernandez-Figares *et al.*, 2002).

I. Specifications of the novel food (NF)

As reported by the applicant betaine is planned to be marketed under three different brand names (Betafin BF20, Betafin TMG20 and Betafin AP). Product specification of Betafin BF20 and TMG20 are presented, both products corresponding to a molecular weight 117.15, formula $(CH_3)_3$-N^+-CH_2-COO^-, and purity minimum 99%. Product specification of Betafin AP (monohydrate form of edible and pharmaceutical quality, molecular weight 135.16, formula $(CH_3)_3$-N^+-CH_2-COO^- x H_2O and purity minimum 99%) and analytical documents from the five last Betafin AP batches are also presented. Analytical methods were provided.

Anhydrous betaine and betaine monohydrate have similar physicochemical characteristics, and similar chemical structure except for the water molecule which is attached to the carbonyl

group in monohydrate form. Both forms do not differ practically in nutritional, microbiological or toxicological properties.

Betaine contains very small amounts of chloride, sulphate and heavy metals. Trace analysis show very small amounts of PCB, PAH and dioxins and no traces of pesticide have been detected. Betaine does not contain methanol, ethanol or isopropanol (limits of detection were 5.0, 2.5 and 0.5 ppm, respectively).

In summary, the residues are considered insignificant.

Proposed betaine conforms to product specifications, as it has been checked in samples obtained from production on an industrial scale and which are representative of the samples to be marketed.

II. Effects of the production process applied to the NF

Betaine extraction from sugar beet involves: a) production of sugar beet molasses from sugar beet (washing, slicing, extracting with water, treatment with calcium hydroxide and carbonating with carbon dioxide followed by concentration into thick syrup and sugar being crystallized out); b) extraction of betaine rich fraction from sugar beet molasses (by liquid chromatography separation), and c) refining and crystallization. Betaine can be crystallized from sugar beet molasses either in anhydrous form or as betaine monohydrate depending on crystallisation parameters used.

When betaine is crystallized sodium hydroxide and formic acid (which are used to adjust the pH) stay in mother liquor. Isopropanol used in crystallization evaporates during crystallization. Betaine preparations are highly hygroscopic. The stability of the products has been ensured by packaging them in moisture-resistant, food quality material. A study report shows that anhydrous betaine remains stable over a period of 3 years when stored at room temperature and in original packaging.

Stability of betaine has also been studied in different conditions. Pure betaine (anhydrous) starts to decompose at temperature higher than 245 ºC. However, in food processes, the internal temperature of those foods that are intended to be enriched with betaine do not rise above 200 ºC suggesting that decomposition of betaine is not to be expected. The same is applicable with respect to betaine monohydrate form as first water will be evaporated thus betaine monohydrate would convert to anhydrous betaine.

The applicant has tested betaine crystals during heat treatment (80, 120 and 200 ºC for 1, 5 or 24 hours) showing stable betaine levels in all temperatures and treatment times.

It is considered by the applicant that decomposition products in foods containing betaine are formed only after very long term exposure to temperatures above 120 ºC, circumstances that are not used in the production process of applied betaine enriched foods. The applicant has also done some thermostability tests with 35% and 50% betaine liquids and checked the stability of betaine in foods. It was shown that concentration of betaine added to cookies (and baked at 154 ºC in a continuous oven) and brownies (baked at 177 ºC in a revolving oven) is similar to that analysed after the food processing.

Hazard analysis critical control point (HACCP) and other internal quality management programs have been established by the applicant to ensure good manufacturing practice (GMP).

III. History of the organism used as the source of the NF

Betaine is produced from sugar beet (*Beta vulgaris sp*), which has a long history as a food source and in the production of sugar in the EU. Sugar molasses from which betaine is isolated is well known as a food ingredient. Betaine has been produced on a commercial scale since 1979, and food grade betaine has been produced since 1983 to be sold in Japan and Korea by using the process proposed by the applicant. Production processes of betaine-enriched foods are also traditional.

IX. Anticipated intake and extent of use of the NF

Betaine is intended for use in drinks, cereal products, confectionary (sweets) and dairy products. The initial application was that betaine would be added to foods that are already on the market with the following maximum betaine content: drinks (2%), sweets (6.7%), cereal products (4%) and milk-based products (2.7%). However, following the initial assessment and the questions raised by different Member States, the applicant is willing to restrict the use of betaine as an ingredient to certain sub-classes of foods groups:

1. Beverages (maximum 1.2%): mineral water, soft drinks for adults and juices (coffee or alcohol drinks will not be enriched);

2. Confectionary (maximum 6.7%): chewing gum, hard candies and strong-flavoured pastilles (the chocolate and wine gum type sweets will not be enriched);

3. Cereal products (maximum 4.0%): breakfast cereals, snack bars and biscuits (bread, buns, cakes and pastries will not be enriched);

4. Dairy products (maximum 2.7%): cheese and yogurt (milk and buttermilk will not be enriched).

The applicant proposes that adults, old people and particularly persons who are otherwise healthy but are running a risk of heart disease should be target groups for the products concerned.

Betaine is intended for supply to food manufacturers. The applicant recommends a total daily intake of 4 g betaine and assumes that food manufacturers will inform consumers of this recommendation by means of appropriate labelling.

The products are not intended for children or pregnant women or breast-feeding mothers, and for that reason, the applicant has not considered it necessary to make a safety evaluation especially with a view to these groups of consumers. The applicant emphasizes that products potentially consumed by children (like soft candies, chocolate, ordinary milk, bread and cakes and soft drinks especially targeted for children) that were initially proposed are now excluded.

The applicant presents the average intakes of several concerned traditional foods in four European countries, showing figures (g/person) varying from country to country for beverages (171 to 356, not including tap water), confectionary (2 to 13), cereal products (24 to 62) and dairy products (36 to 82). High intake levels (mean intake plus 2 SD) of traditional counterparts of potential betaine-enriched foods were also calculated. Betaine intake (g/day) estimated from both average/high intakes were: 4.5/15.2 (beverages), 0.5/2.8 (confectionary), 1.6/7.0 (cereal products) and 1.6/7.0 (dairy products), resulting in cumulative intakes of betaine of 8.2 g/day (average) or 32.0 g/day (high intake).

X. Information from previous human exposure to NF or its source

Exposure to betaine is not new. Intake of betaine is from conventional foodstuffs containing betaine or containing choline or choline-derivatives. It is a natural component in a number of food products. Dietary sources of betaine include fish, beets, legumes, and wheat flour (Sakamoto *et al.*, 2002). The dossier compiles analysis for betaine in more than 50 foods, and it is estimated that betaine intake from natural sources may range from about 0.5 g (average) to 2.5 g for a high seafood diet. A high intake of 2.5 g/day for a diet high in whole wheat and seafood has also been reported (Craig, 2004).

While the main nutrients involved in controlling homocysteine levels are methionine, folic acid, vitamin B_6, and vitamin B_{12}, betaine has been reported to be helpful in some people whose elevated homocysteine levels did not improve with supplementation of the named vitamins. Nevertheless, betaine supplementation (6 g/day) has been shown to lower plasma homocysteine concentrations also in healthy adults (Olthof *et al.*, 2003; Steenge *et al.*, 2003).

According to the applicant, in the EU food supplements with betaine in the form of the hydrochloride salt have been on the market since 1982, with a daily recommended dose level varying from 7 to 324 mg. In the USA betaine has been sold as a dietary supplement since the 1960's. Recommended dose levels range from 0.5 to 1.5 g/day. However, there are no data about the effects of using these supplements.

Several genetic disorders of homocysteine degradation or homocysteine remethylation are known which lead to homocysteine accumulation in the body. Betaine supplements are used to lower levels of homocysteine in individuals with these inherited disorders (Finkelstein and Martin, 2000).

According to the applicant, betaine was approved as an orphan drug in 1966 by the FDA as effective for the treatment of homocystinuria. Typical dosage is 6 g/day, up to 20 g/day. It was registered later in Canada and Australia. However, information about the safety assessments made during this registration procedure was not provided by the applicant. Anhydrous betaine has received orphan drug designation in the EU, but has not yet received marketing authorisation.

XI. Nutritional information on the NF

The betaine-enriched foods are produced using the same ingredients as their traditional counterparts except for betaine. The only intended nutritionally significant difference between

enriched and non-enriched foods is the effect of high betaine ingestion, namely the reduction of blood homocysteine concentrations.

Since anhydrous betaine converts to the monohydrate form when relative humidity is more than 7% there are no difference nutritional properties of both forms, which are equally soluble in the gastrointestinal media.

Kinetics and metabolism

The main metabolic reaction of betaine is probably the transfer of a methyl group from betaine to homocysteine via the enzyme betaine-homocysteine methyl transferase thus forming methionine which is subsequently converted to S-adenosylmethionine (SAM), which serves as a methyl donor in a large number of biochemical reactions (Finkelstein, 2000; Schwahn *et al.*, 2003a), including the formation of nucleic acids, proteins and lipids. There are strong interrelationships between betaine and the metabolism of methionine, homocysteine, folate and vitamin B_{12}, as well as the metabolism of choline. They all carry and/or donate methyl groups, and are thus involved in essential biochemical processes. The role of betaine as a source of methyl groups is important for liver, kidneys and most other tissue functions e.g., in cellular replication, detoxification reactions and others. Betaine is a metabolite of choline. After ingestion, betaine is absorbed into the enterocytes and then released to the portal circulation and carried to the liver where significant first pass extraction and metabolism of betaine occurs. Betaine plasma concentrations change rapidly after ingestion. Results of a pharmacokinetic study of betaine and its metabolite dimethylglycine (DMG) in healthy subjects and in three patients with homocystinuria (Schwahn *et al.*, 2003b) showed rapid absorption (t½, 0.3 hours) and distribution (t½, 0.6 hours) of betaine; a maximum concentration was reached at 0.9 hours. The elimination half life was 14.4 hours. DMG concentrations increased significantly after betaine administration and accumulation occurred to the same extent as with betaine. Distribution and elimination kinetics in homocystinuric patients appeared to be accelerated. Elimination half-life increased during continuous dosing over 5 days. Most of dietary betaine is metabolised and not excreted intact (Schwahn *et al.*, 2003b).

Human circulating betaine levels appears homeostatically controlled within a narrow range for each individual, within typical betaine plasma concentrations of about 20-60 μmol/L (Lever *et al.*, 2004), individual set points remaining stable for years and plasma levels largely maintained for several hours after acute diuretic or antidiuretic stresses (Lever *et al.*, 1994 and 2004).

XII. Microbiological information on the NF

The new ingredient gives no reason for concern with regard to microbiological hazards due to the manufacturing processes. The eventual NF would be expected to be as safe as traditional foods from a microbiological perspective.

XIII. Toxicological information on the NF

Acute toxicity

The acute oral toxicity of betaine was studied in CD rats. Under the conditions of the study, the LD_{50} was about 11 g/kg body weight for both males and females.

Subacute and subchronic toxicity

After an initial 14-day range finding study, groups of 20 male and 20 female Sprague-Dawley rats received 0, 1, 2 or 5% betaine in the diet, equivalent to about 0, 800, 1600 or 4000 mg/kg body weight/day in males and 0, 900, 1800 or 4400 mg/kg body weight/day in females, for a period of 90 days (TNO BIBRA, 2001a). Main findings were dose-related increases in liver weight, hepatocellular microvacuolation in both males and, more pronounced, in females, dose-related increases in mesenteric lymphnode weight in males and ovary weight in females as well as higher kidney weights in the 5% males and females. In addition, there was a dose-related decrease in mean red cell volume and haemoglobin together with a dose-related increase in red blood cell and platelet counts as well as dose-related increases in gamma glutamyltransferase and alkaline phophatase levels in rats of both sexes.

The changes in the liver were assumed to be due to an effect on the intermediary metabolism. Signs of hepatic necrosis or bile duct hyperplasia were not seen. Nevertheless, a NOAEL could not be established, as most of the changes were seen even at the lowest dose level.

In order to reproduce the findings of the 90-study, appearing to be more severe in females, and to investigate the recovery from such effects, groups of 20 female Sprague-Dawley rats were fed betaine in the diet at 0, 1, 2 or 5% for 28 days (TNO BIBRA, 2001b). Ten animals of each group were taken for necropsy on day 28 and the remainder received control diet for a further 28 days. The treatment period caused similar hepatic changes to those seen in the previous study over a 90-day period. The changes were, in general, largely reversible over the 28-day recovery period. “Pale livers” at necropsy and hepatocellular vacuolation were still present in a few animals of the 2% and 5% groups after the reversal period, however.

To establish a NOAEL for betaine based on liver triglyceride accumulation, serum chemistry and blood profile, an additional feeding study, not complying with OECD and GLP requirements, was carried out (Hayes, 2002). Groups of 8 three-week old female Sprague-Dawley rats received a diet with 0, 0.5, 0.75, 1 and 5% betaine for 28 days and groups of 5 female rats were fed 0 or 5% betaine for 90 days. The composition of the diet was different from those in the studies mentioned above.

After 28 days, liver and kidney weight was increased at 5%. The hepatic triglyceride pool was reduced in all test groups and the protein content in the liver was elevated at 5% (other dose groups were not studied). Liver histology was studied with only 3 livers from each of the 0, 1 and 5% groups, resulting at 1% in the same lipid score as in the control group and a reduction to about 1/3 of normal control values on the 5% betaine diet. After 90 days, no differences in liver weight, hepatic triglycerides and protein were observed. The relative weight of perirenal adipose tissue was more than doubled on the 5% diet. In the 90-day groups, livers were not examined histopathologically. The results of plasma and serum chemistry and haematology suffer very much from large variations between individual rats and are only of limited value.

Genotoxicity

Betaine monohydrate was not mutagenic in the Salmonella reverse mutation assay, when tested in strains TA 1535, TA 1537, TA1538, TA 98 and TA 100 up to 5000 µg/plate with and without metabolic activation (S-9 mix). It did not induce micronuclei in the bone marrow of CD-1 mice orally dosed with up to 2000 mg/kg body weight and was not clastogenic, when tested *in vitro* with human lymphocytes at concentrations up to 10 mg/mL in the absence or presence of metabolic activation.

Chronic toxicity, carcinogenicity and reproductive and developmental toxicity

No data available.

Other animal studies

Betaine did not show an eye irritating potential in rabbits and did not cause skin sensitisation in guinea pigs. In a human patch test, it was not a skin irritant.

Human clinical studies

The applicant refers to over 40 published human clinical studies, involving about 700 individuals. Betaine dose levels ranges from 1.5 to 30 g/day, 6 g/day being the most common dose, and period of betaine intake ranging from 1 day to 16 years.

About 90% of the studies were conducted in different types of patients. There was no evidence of organ toxicity, and adverse reactions to betaine were minimal, including incidental reports of nausea, diarrhoea and gastrointestinal distress. However, undesirable side-effects were not systematically explored in these studies.

Given the questions raised by various MS on potential adverse effects of betaine on liver function and haematology in humans, this issue has been readdressed by the applicant. Results have been summarized by the applicant (Table 1). No adverse effects were reported.

Two recent studies have explored the effects of betaine in healthy adults (Brouwer *et al.*, 2000; Schwab *et al.*, 2002). In the first of these studies (Brouwer *et al.*, 2000), 6 g betaine supplementation were given for 3 weeks. As a result, betaine slightly decreased plasma homocysteine levels and no adverse effects were detected, but this study was not placebo-controlled.

The aim of the second study in healthy humans (Schwab *et al.*, 2002) was to examine the effect of betaine supplementation on body weight, body composition, plasma homocysteine concentrations, blood pressure, and serum total and lipoprotein lipids. It involved 42 obese subjects treated with a hypoenergetic diet, randomly assigned to a betaine-supplemented group (6 g/day) or a control group given placebo for 12 weeks. Body weight, resting energy expenditure, and fat mass decreased significantly in both groups with no significant difference between the groups. Plasma homocysteine concentrations decreased in the betaine group. The conclusion was that a hypoenergetic diet with betaine supplementation (6 g daily for 12 weeks) decreased the plasma homocysteine concentration but did not affect body composition more than a hypoenergetic diet without betaine supplementation (Schwab *et al.*, 2002).

However, serum total and LDL-cholesterol concentrations were higher in the betaine group than in the control group.

In a study on 7 subjects suffering from non-alcoholic steatohepatitis and treated with 20 g betaine/day for 1 year it was concluded that betaine is a safe and well tolerated drug that leads to a significant biochemical and histological improvement in these patients (Abdelmalek *et al.*, 2001).

Table 1. The effect of betaine supplementation on haematology, blood chemistry, liver and renal function in human studies as summarized by the applicant

Authors	Length of the study	Subjects completing the study	Betaine doses	Effects on haematology, blood chemistry, liver and renal function tests
Abdelmalek *et al.*, 2001	1 year	7 subjects suffering from non-alcoholic steatohepatitis (NASH)	20 g/day	No adverse effects in haematology or blood chemistry (authors do not give the actual data) betaine supplementation led to significant improvement of the NASH
Brena *et al.*, 1993	1-2 years	3 patients with hyperhomocystinuria	6 g/day	No disturbances in hepatic, renal or bone marrow functions (authors do not give the actual data)
Dudman *et al.*, 1996	Up to 13 years	10 patients with pyridoxine-resistant homocystinuria	2 x 25 mM/day	Renal and liver function tests and haematology remained normal during the betaine treatment (authors do not give the actual data)
Gahl *et al.*, 1988	Cross-over of 2 years	5 patients with pyridoxine-resistant homocystinuria	3 g (b.i.d)	Blood analysis did not suggest any renal, hepatic or haematological complications on betaine therapy (authors do not give the actual data)
Schwab *et al.*, 2002	18 weeks	22 dieting study subjects with 20 controls	6 g/day	No significant differences in liver enzymes, plasma, glucose, serum TSH or creatinine compared to the control group (data shown in the article)
Wilcken *et al.*, 1983	8+3 months	10 patients with homocystinuria	8-9 g/day	No abnormalities in hepatic, renal or bone marrow function (these were tested at each outpatient visit, but data was not shown). In addition serum B_{12} levels were unchanged

Dudman et *al.* reported that long-term betaine supplementation of 10 patients, who had pyridoxine-resistant homocystinuria and gross hyperhomocysteinemia due to deficiency of cystathionine β-synthase activity, caused a substantial lowering of plasma homocysteine, which has been maintained for periods of up to 13 years (Dudman *et al.*, 1996).

Two reports of serious cerebral oedema in betaine-treated children with homocystinuria due to cystathionine β-synthase deficiency not responsive to vitamin B_6 have been published.

Betaine elimination from the therapeutic regimen reversed the symptoms in both. One case concerned a 5 year old boy who developed cerebral oedema necessitating decompressing craniotomies, 4-6 weeks after the initiation of betaine administration (150 mg/kg body weight/day) in addition to a low-methionine diet. Plasma and cerebrospinal fluid levels of methionine markedly increased (Devlin *et al*., 2004). The other case was a 10 years old child, which after three months of betaine supplementation (6 g/day corresponding to 200 mg betaine/kg body weight/day) showed headaches and signs of increased intracranial pressure, confirmed by CT and MRI (Yaghmai *et al.*, 2002). One reason for this adverse effect could be an intracellular accumulation of betaine in the brain due to a variant in the transporter that carries betaine into cells (Devlin *et al*., 2004).

Gahl *et al.* (1988) reported that five pyridoxine non-responsive homocystinuric patients aged 5 to 32 years were treated with oral betaine, 3 g twice a day in a double-blind, placebo-controlled, two-year crossover study of its effect on bone mineralization. Betaine therapy significantly reduced mean plasma homocystine with variable increases in plasma methionine and no adverse effects.

Wilcken *et al.* (1983) studied 10 patients with cystathionine β-synthase deficiency that was not responsive to pyridoxine and one patient with homocystinuria due to a defect in cobalamin metabolism. They were treated with 6 g daily of betaine added to conventional therapy, to improve homocysteine remethylation. All patients had a substantial decrease in plasma total homocysteine levels and an increase in total cysteine levels. Changes in plasma methionine concentrations were variable. Fasting levels of plasma amino acids became normal in two patients, and in six there was immediate clinical improvement. No unwanted effects were observed. The authors concluded that treatment of homocystinuria that is not responsive to pyridoxine and of disorders of homocysteine remethylation should include betaine in adequate doses to ensure maximum lowering of elevated plasma homocysteine levels (Wilcken *et al.*, 1983).

Two additional studies on betaine efficacy have been published (Olthof *et al.*, 2003; Steenge *et al.*, 2003). Four groups of 19 healthy subjects ingested three doses of betaine or placebo daily for 6 weeks. A methionine loading test was performed on day 1 of betaine supplementation, and after 2 and 6 weeks of betaine supplementation. Significant changes in fasting plasma homocysteine after 6-week daily intakes of 1.5, 3 and 6 g of betaine were 12%, 15%, and 20% less than in the placebo group, respectively. Furthermore, the increase in plasma homocysteine after methionine loading on the first day of betaine supplementation was 16%, 23% and 35% less than in the placebo group, respectively, and after 6 weeks of supplementation was 23%, 30% and 40% less, respectively. Thus, doses of betaine in the range of dietary intake reduce fasting and postmethionine loading plasma homocysteine concentrations, supporting the idea of the authors that betaine-rich diet might therefore lower cardiovascular disease risk (Olthof *et al.*, 2003).

The effect of daily betaine supplementation, compared with both folic acid and placebo, on plasma concentrations of total homocysteine after an overnight fast and after methionine loading in men and women with mildly elevated homocysteine, has been investigated (Steenge *et al.*, 2003). Groups of twelve subjects ingested 6 g betaine, 800 µg folic acid with 6 g placebo or 6 g placebo each day for 6 weeks. A methionine-loading test (i.e., ingestion of 100 mg L-methionine/kg body mass) was performed before and after 6 weeks of supplementation. Fasting plasma homocysteine significantly decreased (relative to the change in the placebo group) 1.8 µmol/L in the betaine group and by 2.7 µmol/L in the folic acid

group. Furthermore, betaine suppressed the total area under the plasma homocysteine-time curve after methionine loading by 221 µmol compared with placebo, whereas folic acid had no effect. In conclusion, betaine appears to be highly effective in preventing a rise in plasma homocysteine concentration after methionine intake in subjects with mildly elevated homocysteine (Steenge *et al*., 2003).

DISCUSSION

Safety. Animal and human studies

Results of the animal studies do not prove satisfactorily that betaine is safe. Subacute and subchronic toxicity studies have clearly shown that the liver is the main target organ in rats. The dose-related effects, more pronounced in females, included increased liver weight, hepatocellular vacuolisation and increased serum levels of liver enzymes. The changes were largely reversible. They were observed, however, at all tested dose levels, even after a short treatment period of 14 days. Therefore, a NOAEL could not be established. Another study, in which betaine was given to female rats in a diet of different composition and in which "no apparent abnormalities of clinical importance" were reported, does not comply with OECD and GLP requirements for studies.

As the available studies do not allow the derivation of a NOAEL for betaine and data on reproduction and developmental toxicity are lacking, an acceptable daily intake cannot be established from animal data.

The data submitted refer to a number of published clinical studies using betaine. Irrespective of some few reported non-severe problems (nausea, diarrhoea or gastrointestinal troubles) the general agreement is that betaine is well tolerated at dosages up to 30 g per day and no differences between tolerance to betaine of adult and old people have been found. However, the conclusion of the applicant that regular consumption of up to 30 g supplemental betaine does not involve a health risk appears feebly based. Most of the studies were done in patients and adverse side-effects were not systematically investigated. However, two case reports describe a serious adverse effect in two children with homocystinuria not responsive to vitamin B_6 with betaine doses of 150 and 200 mg/kg body weight per day. Other studies in adults used betaine doses up to 6 g/day (corresponding to about 100 mg/kg body weight/day) for relatively short periods (3-12 weeks). The metabolic effects of betaine in presence of disorders of sulphur amino acid metabolism have not been thoroughly explored and serious adverse reactions cannot be excluded even at doses that are otherwise well tolerated by healthy individuals.

The data submitted contain the results of only one placebo-controlled randomized study in healthy humans (Schwab *et al.*, 2002) and reported no side-effects at an exposure of 6 g per day for 12 weeks.

The effects of betaine supplementation on cholesterol metabolism have not been clearly elucidated (McGregor *et al.*, 2002; Schwab *et al.*, 2002). One study (4 g betaine per day for 3 months in 36 patients with chronic renal failure) reported that serum lipids were increased although the ratio total to HDL cholesterol was unchanged (McGregor *et al*. 2002). In another study, obese volunteers (14 men, 28 women) on an energy-restricted diet were randomly assigned to a betaine-supplemented group (6 g/day) or a control group given placebo for 12

weeks. Both serum total and LDL-cholesterol concentrations were significantly higher in the betaine group than in the control group. The absolute decrease in total cholesterol and triglyceride levels compared to baseline was the same in both groups during the study (by 0.3 mmol/L and 0.2 mmol/L, respectively) (Schwab *et al.,* 2002).

It is noted that the animal studies showed certain effects of betaine on cholesterol levels, which were in opposite directions in male and female rats. In any case, the Panel considers that if blood levels of cholesterol are affected adversely this should be considered an important adverse effect, which is opposed to the beneficial effect claimed by the applicant.

The Panel shares the opinion of some MS that it is difficult to assess the significance for consumer health of the observed effects in animal and clinical studies. Statements by the applicant that the adverse changes observed in the animal studies are "generally" or "mainly" reversible are not reassuring enough. In particular, reversibility may not be so relevant if betaine is being taken regularly to achieve a persistent reduction in homocysteine levels.

In summary, because the available human data do not satisfactorily exclude the possibility of risk from the intended intake of betaine, additional studies with rats and another species should be carried out, in order to establish NOAELs. In such studies, the influence of the diet should be clarified.

Intended use and anticipated intake

The Panel considers that the range of foods to which betaine is intended to be added is still too broad. It is extremely difficult to estimate betaine intake also because the applicant plans to add this substance not only to individual foodstuffs but to a wide range of food categories.

There is also a concern that young people might be attracted to enriched confectionery and soft drinks. It appears contradictory to add betaine to soft drinks and confectionary as these are consumed in far greater quantities by children than by the target groups.

Absorption of choline and derivatives

A plausible response has been given by the applicant to questions raised by MS authorities on the betaine potential to affect absorption of choline and its derivatives: this effect is not supported by the existing physicochemical differences and different transport systems between the concerned compounds and betaine, as well as because physiologically important choline derivatives are synthesized in the body by demand rather than obtained from the diet.

Glutathione homeostasis

A MS raised a potential concern on some data about the effects of betaine on glutathione homeostasis. As addressed by the applicant, some experimental studies have investigated this aspect (Kim et *al.*, 1998 and 2003; Kim and Kim, 2002). First it was reported that betaine enhances the toxic response in mice when injected intraperitoneally 1 to 4 hours before chloroform administration (Kim *et al.*, 1998). However when betaine was given 24 hours prior to chloroform it decreased chloroform toxicity (Kim *et al.*, 1998). In a more recent study it was found that betaine when administered in drinking water (1%) attenuated hepatoxicity induced by bacterial lipopolysacharide to rats (Kim and Kim, 2002).

It has been suggested that betaine enhances metabolic reactions in the methionine cycle, but inhibits cystathionine and cysteine synthesis, leading to a decrease in supply of cysteine for glutathione synthesis. However effects observed in mice and rats are time-dependent and reduction in glutathione is subsequently reversed, what is suggested to be due to induction of cysteine synthesis and glutamate cysteine ligase activity (Kim *et al.*, 2003).

Orendac *et al.* (2003) reported that glutathione levels in plasma were not decreased in nine patients with homocystinuria due to cystathionin β-synthase deficiency despite a substantially blocked transsulphuration pathway with undetectable cystathionine and decreased total cysteine levels in plasma.

Kinetics of methylation

The possibility of disturbances in the kinetics of methylation at doses as small as 3 g betaine per day when administered to healthy males have been described (Storch *et al.*, 1991) suggesting that the excess betaine intake may increase the dietary requirement for methionine. Although the data are quite limited it could point to a potential concern with betaine consumption from fortified foods in certain population groups which may be at risk of nutritional deficiency if they consume betaine-enriched foods, due to an increased requirement for dietary methionine. As raised by a MS, in the absence of appropriate studies (i.e. on individuals consuming a low protein or low methionine diet) this potential risk is not assessable.

No information on betaine effects on DNA methylation, e.g. in relation to epigenetics (Santos and Dean, 2004) have been provided.

Vitamin B_{12} deficiency

The enzyme betaine methyltransferase is peripherally distributed but not present in the brain where the methionine synthetase (B_{12} dependent) pathway principally governs methylation of homocysteine. Betaine therefore has the potential to correct peripheral methylation, but not central methylation in vitamin B_{12}-deficient people and therefore to mask the symptoms of deficiency. This effect cannot be ruled out in the absence of clinical studies in individuals with an abnormal vitamin B_{12} status.

CONCLUSIONS AND RECOMMENDATIONS

From its evaluation of the available evidence on the safety of betaine from both animal and human studies the Panel considers that a number of safety questions remain unresolved.

Animal studies showed treatment-related effects that were observed at all tested doses of betaine, the biological or toxicological significance of which have not been satisfactorily clarified. Since the available studies do not allow the derivation of a no observed adverse effect level (NOAEL) for betaine and data on reproduction and developmental toxicity are lacking, an acceptable daily intake cannot be established.

The available data in human studies are insufficient to allow the Panel to rule out the safety concerns raised by the MS.

Therefore, it is concluded that the safety of betaine for the intended use as proposed by the applicant has not been established.

DOCUMENTATION PROVIDED TO EFSA

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