Betaine has also been demonstrated to increase low plasma methionine and S-adenosylmethionine (SAM) levels in patients with MTHFR deficiency and *cbl* defect.

In CBS-deficient patients, large increases in methionine levels over baseline have been observed.

Betaine occurs naturally in the body. It is a metabolite of choline and is present in small amounts in foods such as beets, spinach, cereals, and seafood.

Pharmacokinetic studies of betaine are not available. Plasma levels of betaine have not been measured in patients and have not been correlated to homocysteine levels. However, pharmacodynamic measurements, i.e., monitoring of plasma homocysteine levels, have demonstrated that the onset of action of betaine is within several days and that a steady state in response to dosage is achieved within several weeks. Patients have taken betaine for many years without evidence of tolerance.

INDICATIONS AND USAGE

Cystadane (betaine anhydrous for oral solution) is indicated for the treatment of homocystinuria to decrease elevated homocysteine blood levels. Included within the category of homocystinuria are deficiencies or defects in:

1. cystathionine beta-synthase (CBS),
2. 5,10-methylenetetrahydrofolate reductase (MTHFR),
3. cobalamin cofactor metabolism (*cbl*).

Patient response to Cystadane can be monitored by homocysteine plasma levels (see DOSAGE AND ADMINISTRATION). Response usually occurs within a week and steady state within a month.

Cystadane has been administered concomitantly with vitamin B_6 (pyridoxine), vitamin B_{12} (cobalamin), and folate.

PRECAUTIONS

General

Therapy with Cystadane should be directed by physicians knowledgeable in the management of patients with homocystinuria.

Hypermethioninemia

Patients with homocystinuria due to cystathionine beta-synthase (CBS) deficiency may also have elevated plasma methionine concentrations. Treatment with Cystadane may further

increase methionine concentrations due to the remethylation of homocysteine to methionine. Cerebral edema has been reported in patients with hypermethioninemia, including a few patients treated with Cystadane. Plasma methionine concentrations should be monitored in patients with CBS deficiency. Plasma methionine concentrations should be kept below 1,000 umol/L through dietary modification and, if necessary, a reduction of Cystadane dose.

Information For Patients

1. Shake bottle lightly before removing cap.
2. Measure with the scoop provided.
3. One level scoop (1.7 cc) is equivalent to 1 gram of betaine anhydrous powder. Measure the number of scoops your physician has prescribed.
4. Mix with 4 to 6 ounces (120 to 180 mL) of water, juice, milk, or formula until completely dissolved, or mix with food, then ingest immediately.

Always replace the cap tightly after using. Protect from moisture.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term carcinogenicity and fertility studies have not been conducted on betaine. No evidence of genotoxicity was demonstrated in the following tests: Metaphase Analysis of Human Lymphocytes; Bacterial Reverse Mutation Assay; and Mouse Micronucleus Test.

Pregnancy

Pregnancy Category C. Animal reproduction studies have not been conducted with betaine. It is also not known whether betaine can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Cystadane (betaine anhydrous for oral solution) should be given to a pregnant woman only if clearly needed.

Nursing Mothers

It is not known whether betaine is excreted in human milk (although its metabolic precursor, choline, occurs at high levels in human milk). Because many drugs are excreted in human milk, caution should be exercised when Cystadane is administered to a nursing woman.

Pediatric Use

The majority of case studies of homocystinuria patients treated with betaine have been pediatric patients. The disorder, in its most severe form, can be manifested within the first months or years of life by lethargy, failure to thrive, developmental delays, seizures, or optic lens displacement. Patients have been treated successfully without adverse effects within the first months or years of life with dosages of 6 grams per day or more of betaine with resultant biochemical and clinical improvement. However, dosage titration may be preferable in pediatric patients (see DOSAGE AND ADMINISTRATION).

ADVERSE REACTIONS

Adverse reactions to betaine have been minimal. In a survey study of physicians who had treated a total of 111 homocystinuria patients with betaine, the types of adverse effects and the number of patients experiencing them were as follows:

Nausea	2	"Caused odor"	1
GI distress	2	Questionable psychological changes	1
Diarrhea	1	"Aspirated the powder"	1
Unspecified problem	1		

A few cases of cerebral edema have been reported secondary to severe hypermethioninemia in patients with cystathionine beta-synthase (CBS) deficiency treated with Cystadane. See PRECAUTIONS: Hypermethioninemia.

OVERDOSAGE

In an acute toxicology study in rats, death frequently occurred at doses equal to or greater than 10,000 mg/kg.

DOSAGE AND ADMINISTRATION

The usual dosage used in adult and pediatric patients is 6 grams per day administered orally in divided doses of 3 grams two times per day. Dosages of up to 20 grams per day have been necessary to control homocysteine levels in some patients. In pediatric patients less than 3 years of age, dosage may be started at 100 mg/kg/day and then increased weekly by 50 mg/kg increments. In one study by Matthews[1] et al., pharmacokinetic and pharmacodynamic simulation indicated minimal benefit from exceeding a twice-daily dosing schedule and a 150 mg/kg/day dosage for betaine. Dosage in all patients can be gradually increased until plasma total homocysteine is undetectable or present only in small amounts. Plasma methionine concentrations should be monitored in patients with CBS-deficiency. See PRECAUTIONS: Hypermethioninemia.

The prescribed amount of Cystadane (betaine anhydrous for oral solution) should be measured with the measuring scoop provided (one level 1.7 cc scoop is equal to 1 gram of betaine anhydrous powder) and then dissolved in 4 to 6 ounces (120 to 180 mL) of water, juice, milk, or formula, or mixed with food for immediate ingestion.

HOW SUPPLIED

Cystadane is available in plastic bottles containing 180 grams of betaine anhydrous. Each bottle is equipped with a plastic child-resistant cap and is supplied with a polystyrene measuring scoop. One level scoop (1.7 cc) is equal to 1 gram of betaine anhydrous powder.

NDC 62161-004-20 180g/bottle
DIN 02238526

Store at room temperature, 15° - 30°C (59° - 86°F).

For questions of a medical nature in the U.S. or Canada, call 1-888-8ORPHAN (1-888-867-7426).

Cystadane® can be ordered by calling Orphan Medical, Inc. Customer Service at 1-800-359-4304 or by contacting your local wholesaler.

Distributed in the U.S. by
Orphan Medical, Inc.
Minnetonka, MN 55305

Distributed in Canada by:
Orphan Medical, Inc.
Scarborough, Ontario
M1H 2W4

Revision Date: Nov 2005
Part No. 4040155

---

[i] Matthews A, Johnson TN, Rostami-Hodjegan A, Chakrapani A, et al. An indirect response model of homocysteine suppression by betaine: optimizing the dosage regimen of betaine in homocystinuria. Br J Clin Pharmacol 2002; 54:140-146.

SCIENTIFIC DISCUSSION

1. Introduction

Homocystinuria is a serious life-long disease and is associated with a high morbidity and mortality. Treatment aims to reduce homocysteine accumulation and to restore the transmethylation capacity by normalising the concentrations of S-adenosylmethionine (SAH) and S-adenosylhomocysteine (SAM).

Homocystinuria is an inherited disorder of the metabolism of the amino acid methionine leading to accumulation of homocysteine in the blood and urine. This is due to a dysfunction in one of the metabolic pathways responsible for transulfuration and remethylation of homocysteine. It is considered the second most common inborn error of amino acid metabolism after phenylketonuria. The major clinical manifestations include mental retardation, dislocation of the optic lentis (ectopia lentis), skeletal abnormalities and a tendency to thromboembolic episodes. The estimated incidence of homocystinuria due to Cystathionine beta-synthase (CBS) deficiency is 1 in 335 000 births worldwide, with marked regional variations.

The aim of the treatment of homocystinuria is to normalize homosysteine levels by several ways in varying combinations such as: boosting residual enzyme activity with vitamin B6, vitamin B12 and folates, reducing load on the metabolic pathway affected with low-methionine diet (CBS deficiency), supplementation with the deficient products downstream of the enzyme abnormality (cysteine for CBS deficiency and methionine for impaired remethylation), using the alternative pathway to eliminate the toxic substrate i.e. pharmacologic treatment with betaine which remethylates homocysteine to methionine.

Betaine hydrochloride is a drug product available over-the-counter used to acidify the gastric juice in patients with maldigestion due to achlorhydria. Betaine dihydrogencitrate is available as a drug product in Germany (Flacar®, Fa. Schwabe) and recommended for fatty liver and other liver diseases in amounts of 2-4 grams per day.
Betaine anhydrous (Cystadane) is given to patients with homocystinuria in Europe on a compassionate use basis. Betaine anhydrous has been obtained from chemical companies to treat patients with homocystinuria for many years.

These forms of betaine dissolve easily and completely at physiologic pH in the gastrointestinal tract. Due to its molecular structure and rapid absorption kinetics it is generally accepted that the bioavailability of oral betaine is high, although this has never been formally evaluated. The bioavailability of all betaine salts should theoretically be similar although this has also not been formally tested.

Currently there is no treatment to correct the basic genetic causes of homocystinuria. Consequently, therapy is directed at correcting the biochemical abnormalities of these disorders.

Betaine anhydrous was granted orphan medicinal product status for treatment of the orphan condition homocystinuria in July 2001.

Betaine (trimethyl glycine) acts by remethylation of homocysteine to methionine, and thereby reduces levels of homocysteine. Betaine is found naturally in many foods, including sugar beets, however levels founds are insufficient to achieve reduction in homocysteine levels in patients suffering from the condition. Patients are usually treated with a dose of 3g twice a day of betaine.

Cystadane (betaine) is formulated as an oral powder allowing for weight-adapted dosage in the important patient group of children.

The Applicant has submitted a complete mixed application (containing own data from the company and bibliographic data) through the Centralised procedure.

Cystadane is indicated for the treatment of homocystinuria, involving deficiencies or defects in:

- Cystathionine beta-synthase (CBS),
- 5, 10-methyleneterahydrofolate reductase (MTHFR),
- Cobalamin cofactor metabolism (cbl).

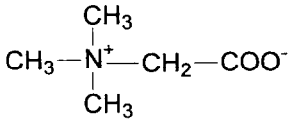
2. Quality aspects

Introduction

Betaine is a well known, naturally occurring drug substance that can be isolated from sugar beets. The drug product is an oral powder consisting of 1 g of betaine (betaine free base) without any excipients.
It is packaged in 300 ml High-Density polyethylene (HDPE) bottle with polypropylene (PP) child resistant caps and three polystyrene spoons as measuring devices for the administration of the product.
It is proposed as an orphan drug for the treatment of homocystinuria.

Active Substance

Betaine anhydrous is not described in the European Pharmacopoeia and scientific data have been submitted in the form of The Active Substance Master File (ASMF). The active substance is in the anhydrous form and the structural formula is provided below:



Betaine anhydrous exists either as white crystals or a crystalline powder with a weak characteristic odour. It is freely soluble in water. It has melting point range of 301-305°C and a bulk density of 0.71g/l. The pH of a 1% aqueous solution was found to be 5.0 - 8.0. It is hygroscopic.

Manufacture

Description of Manufacturing Process and Process Controls

Betaine technical grade is extracted from sugar beet molasses as a result of a purification process.
Betaine occurs with an average abundance of 0.2 % - 0.3 % in the common variety of sugar beets (beta vulgaris L) and is a natural key-intermediate of the amino acid and methyl-metabolism in all vertebrates.
Efforts of the sugar industry to increase sugar yields have led to the development of chromatographic processes for large scale desugarization of molasses. Ion exclusion is used to separate sugar and non-sugar fractions of molasses.

The manufacturing process can be summarised as follow:
1: Pre-process
Raw material – dilution – filtration – twice [ion exclusion chromatography – concentration by evaporation of the Betaine fraction] – separation by centrifugation – drying to Betaine technical grade.

2: Betaine process
Starting material - dissolution with distilled water - activated carbon treatment – filtration, microfiltration - crystallisation by evaporation – separation by centrifugation – vacuum drying – sieving – packaging – weighing.

Sections related to control of materials, control of critical steps and intermediates, process validation and manufacturing process development are suitably detailed in the closed part of the ASMF.

The structure has been satisfactorily elucidated by elemental analysis and spectral analyses i.e. X-ray powder diffraction, UV, IR, and ^{1}H- NMR spectroscopic analysis. The solid state is characterised by refractive index, thermal analysis, thermogravic analysis, and microscopic appearance.

Potential impurities arising from the manufacturing process are amino acids and sugars. They are quantitatively removed during the purification process. Their presence is controlled by adequate analytical methods.
Appropriate data and discussion regarding potential degradation products have been included.
Residual solvents testing was not deemed necessary since only water was used during the manufacturing process of betaine.

- Specification

Since betaine anhydrous is not described in any pharmacopoeia, an internal monograph has been developed by the ASMF holder as well as by the applicant, Orphan Europe. Both specifications are detailed. They differ slightly but are both acceptable.

Appropriate specification for betaine includes parameters such as appearance, identification, pH, loss on drying, assay, related substances, particle size, bulk volume, microbiological quality. Analytical methods are in accordance with Ph.Eur.

Impurity limits have been adequately justified by batch analysis, stability studies, toxicological and clinical studies.

Analytical methods to control the active substance such as test on foreign matter, assay and related sugar impurities (HPLC) and other amino acids (AAA), identification (IR), limit tests for chloride, sulphate, iron and heavy metals, pH, loss on drying, sulphated ash and total viable aerobic count have been suitably described and validated by both the applicant and the ASMF holder in accordance with ICH guidelines.

Data are provided for 3 batches of active betaine anhydrous tested by the ASMF holder and 3 batches tested by the applicant. Results are in line with the respective specification and confirm the consistency and uniformity of the process.

Stability

Stability Summary and Conclusions

Stability studies were carried out by the ASMF holder on 3 batches kept in the commercial container at room temperature (not ICH conditions, but temperature and humidity were continuously monitored). Criteria tested included: appearance, odour, loss on drying, assay, pH, transmittance, related substances, total viable aerobic count and yeasts and moulds.
Results were provided up to 6 years, the active substance remained very stable except few out-of-spec results found after 3 years for loss on drying. The ASMF holder has committed to conduct post-approval stability studies under ICH conditions.

Stability studies were performed by the applicant on 3 production-scale batches kept in a smaller version of the commercial container under ICH conditions (up to 24 months at 25 °C / 60% RH and 6 months at 40 °C / 75% RH). Criteria tested included: description, identification, appearance of constituted solution, pH, loss on drying, saccharide & amino acid impurities, assay, and microbial limits. All results remained within the specification after long-term and accelerated storage except 2 results (loss on drying test) adequately justified. No saccharide or amino acid impurities were detected, and microbial results were within set specifications.

Results showed that forced degradation studies (oxidation, acid and alkali hydrolysis, elevated temperature and light) did not adversely affect the active substance.
Based on the presented data, the proposed retest period of 2 years by the applicant is considered satisfactory. However, the ASMF holder has committed to conduct post-approval stability studies under ICH conditions.

Medicinal Product

Pharmaceutical Development

Betaine in the anhydrous form is a white crystalline powder. Dissolution studies were omitted since it is freely soluble in water.

It exists as anhydrous, monohydrate and hydrochloride forms. The applicant has justified its choice of the anhydrous form; the hydrochloride was discounted on organoleptic reasoning, and the monohydrate was not chosen due to poor flow properties of the compound.

The applicant has discussed in detail the implications of formation of the monohydrate form, and the effect of humidity and high temperature on the product. Humidity conditions above 50% were found to have a negative impact on the powder with moisture absorbance and deliquescence observed. Consequently filling conditions are maintained below 40% humidity.

The applicant has provided justification for a finished product consisting solely of the active, on the grounds that the drug substance has ideal flow characteristics, is freely soluble in water, has a low angle of repose and the quantity to be consumed by the patient (up to 20 g daily) and this is considered satisfactory.

Adventitious Agents

Potential contaminates originating from the starting material used for synthesis of the drug substance have been convincingly ruled out.

Manufacture of the Product

The manufacturing process has been adequately detailed and consists solely of filling the bottle with the active substance, outer labelling and packaging, QC control and EU release (Orphan Europe, France). An overage has been added for the filling and is adequately justified.

Parameters such as temperature and relative humidity control were identified as critical parameters and controlled during the filling process.

A process validation protocol describing the operations, equipment used and tests to be performed have been provided, as well as validation results of three consecutive batches. All three lots complied with the acceptance criteria.

Product Specification

Appropriate finished product specification at release and end of shelf-life have been provided. It includes parameters such as identification (IR, HPLC), appearance of the constituted solution, odour

testing, loss on drying, Impurity (GCP), assay of betaine (HPLC), uniformity of mass of delivered doses from multidose containers (Ph. Eur. 2.9.27) and microbiological quality (skip testing).
Analytical methods have been described and validated apart from the methods described in Ph.Eur.

Batch analyses are provided for 3 pilot batches of finished product. All results appear to meet the finished product specification. Assay was always above 98%, loss on drying was below 1.0% and no amino acid or saccharide impurities were detected.

The applicant has referred to the section on impurities as per section 3.2.S.3.2 impurities and as the finished product consists only of the active, this is considered satisfactory. Limits and specifications are adequately justified.

Cystadane is packed in a 300cc round, opaque, white, HDPE bottle with a white Polypropylene child resistant closure. Three polystyrene measuring spoons are enclosed in the carton for each bottle. Information on the measuring devices can be found in section 3.2.R regional information and the applicant commits to perform post-approval additional testing and to provide results for the "uniformity of mass of delivered doses".

Sources and specifications of the bottle and cap are specified and supported by certificates of analysis from the finished product manufacturer.
Primary packaging materials such as HDPE and PP are in line with the Ph.Eur. Requirements.

Stability of the Product

Three pilot batches of the finished product stored in the commercial packaging have been placed on stability under ICH conditions (up to 24 months at 25 ± 2°C /60 ± 5%RH and 6 months at 40 ± 2°C /75 ± 5%RH). Results are in accordance with the specification, and in particular assay remains within the range 95 – 105%, and neither saccharide nor amino acid impurities were detected at any time point for for both storage conditions. Based on the available data, a shelf life of 2 years with the precaution "do not store above 25°C" and "keep the bottle tightly closed in order to protect against moisture" can be granted. The applicant commits to perform stability studies under ICH conditions and in accordance with revised specification.

In-use stability studies reflecting the conditions of use of the product were performed. The finished product was stored at ambient temperature and under high humidity conditions (75% RH) since the active substance is hygroscopic. The bottle was opened daily and a sample was withdrawn. Analysis of the remaining powder was performed until 31 days in order to cover the time required when dosages under 6 g/per day are used for children under 10 years of age. Results were all within the proposed specification.

Additional results of stability testing performed on two batches of the finished product, in aqueous solution and in simulated gastric fluid were presented. The quantity of powder (6 g) dissolved was equivalent to a dose of the finished product as per section 4.2 of the SPC. Satisfactory assay results were provided for product solutions over 24 h in water and 2 h in simulated gastric fluid.

The applicant has committed to continue testing of the 3 batches to the end of the shelf life (2 years) and to place 1 batch per year under stability. Any deviation would be reported.

Discussion on chemical, pharmaceutical and biological aspects

Generally, satisfactory documentation has been provided and the minor objections raised in the Day 120 List of Questions and D 180 List of Oustanding Issues were solved during the evaluation procedure. The active substance betaine is well-characterised and the retained specification is acceptable.
Results showed that betaine is stable and stability data support the proposed re-test period.

Regarding the finished product, the manufacturing process is a simple filling process and has been adequately described and controlled. It should ensure a consistent quality for the product. Appropriate specification has been selected for this oral powder.
Stability studies under ICH conditions have demonstrated the good stability of the finished product. Stability data support the proposed shelf-life (before opening and in-use) and storage conditions as defined in the Summary of Product Characteristics.
At the time of the CHMP opinion, there were some outstanding minor quality issues with no impact on the benefit/risk. The applicant undertook to provide with the necessary information as follow-up measures within an agreed timeframe and to submit variations if required following the evaluation of this additional information.

3. Non-clinical aspects

Introduction

The pharmacology and the toxicology sections are based on the applicant's own data and bibliographic data. The applicant performed one primary pharmacodynamic study and four toxicology studies (one acute toxicity testing and three tests for genotoxicity). All other data was of bibliographic origin.

The search for preclinical bibliographic data was performed in two steps.

The first step was conducted in 1995 by the literature search service of the Minnesota Technology's Project Outreach. In this attempt articles on animal studies with betaine from 1967 to 1995 were acquired from the databases MEDLINE, Toxlit and PUBMED. The search was performed for CAS registry "107-43-7" or for "betaine" or "trimethylglycine". 71 articles where considered useful for the "non-clinical development program" at this time point.

The second step was conducted in 2005 with a literature search in several database such as MEDLINE, TOXLINE and BIOSIS for the period 1995-2005. The key words combined terms like "cystadane, betaine, betaine-anhydous, trimethylglycine, N-trimethylglycine, methanaminium, in vitro, animal-study, animal-model, pharmacology, pharmacokinetics, toxicology or toxicity". About 150 articles were identified and 58 were considered useful.

The applicant focused on 45 relevant references.

Pharmacology

Betaine (trimethylglycin, CAS: 107-43-7, Mw: 117.15) is a natural occurring quaternary amine closely related to the amino acid glycine. Its primary function in mammals is within the metabolic cycle of methionine. Catabolism of methionine occurs by transfer of the adenosyl group from ATP to the sulphur group of methionine forming S-adenosylmethionin. Subsequently S-adenosylhomocystein is formed by transfer of the methyl group to an appropriate acceptor such as phosphatidyl ethanolamine. S-adenosylhomocysteine is then hydrolysed to homocysteine and adenosine. Methionine can be regenerated (1) by transfer of the methyl group of methyltetrahydrofolate to homocysteine by homocysteine methyltransferase (MS) (resulting in methionine and tetrahydrofolate) or (2) by transfer of the methyl group of betaine by the betainehomocysteinmethyltransferase (BHMT) (resulting in methionine and dimethylglycine). These reactions constitute the well known activated methyl cycle. In addition homocysteine is an intermediate in the synthesis of cysteine (3). Therefore homocysteine and serine are condensed to cystathionine by the cystathionine synthetase (CBS). Cysthationine is deaminated and cleaved by cystathioninease to cysteine and α-ketobutyrate.

The catabolic pathway of methionine and the role of homocysteine are well established and can be found in several textbooks of biochemistry (Biochemistry; Streyer 1988, third edition). The role of betaine is well defined too. The rationale for developing betaine as treatment for homocysteinuria is reasonable.

Primary pharmacodynamics

Several pivotal primary pharmacodynamics studies were provided which showed that the BHMT-catalysed reaction is inducible in rats, mice and chicks. A second set of experiments in MS-inactivated bats showed that betaine is able to prevent neurological impairment and that betaine is able to stimulate the BHMT dependent pathway under these conditions. A third set of experiments in ethanol fed rats showed that betaine is able to prevent 5-methylterahydrofolate accumulation indicating an activation of the BHMT pathway. In summary pivotal studies were presented which demonstrated that the BHMT pathway can be activated by betaine in several mammalian species.

Secondary pharmacodynamics

Some secondary pharmacodynamics studies demonstrated antioxidative, anticonvulsant, osmolytic function and anti-apoptotic effects of betaine in several models.

In the rat, betaine was demonstrated to protect the liver against ethanol-induced injury and to prevent vitamin A depletion. The antioxidative effect of betaine was related to its ability to increase hepatic SAM (S-adenosylhomocysteine) levels and to prevent fatty liver in ethanol-fed rats.

In rats and mice, betaine (intraperitoneal administration) blocked homocysteine-induced convulsions and antagonised strychnine-, electroshock-, and PTZ- (pentilentetrazole) induced seizures. Following local applications, it also blocked neuronal excitation induced by glutamate and by homocysteine in the rat. Since PTZ is known to block chloride channels and reduce GABAergic inhibition, an interaction is consistent with the physiological effect of betaine as an osmolytic protectant.

The protective function of betaine as an organic osmolyte was demonstrated in the rat.

The increase of superoxide dismutase and catalase activities and the decrease of glutathione peroxidase activity induced by subcutaneous administration of carbon tetrachloride was normalised in the kidney of rats pretreated with betaine. Pre-incubation of rat culture hepatocytes with betaine largely prevented bile acid induced apoptosis. In vivo betaine supplementation in water significantly ameliorated hepatocyte apoptosis following bile duct ligation.

Safety pharmacology programme

No specific studies regarding safety pharmacology (except for CNS parameters) and pharmacodynamic interaction were presented. Non-clinical and human data was submitted regarding the influence of betaine on renal system, CNS and CVS. With respect to the safety pharmacology the data was incomplete.

The applicant had not provided any relevant recent clinical data concerning potential effects on the CVS in particular on the electrophysiology. The influence of betaine on the electrophysiology of cardiac action was not discussed. Since homocysteinuria itself increases the risk for cardiovascular diseases it can be assumed that treated patients are closely monitored by their physicians and potential electrophysiological disturbances would have been reported in the past. Also, taking into account that betaine is a natural constituent of the human nutrition the CHMP found the lack of non-clinical data acceptable.

Pharmacodynamic drug interactions

No specific studies have been presented.

Pharmacokinetics

Betaine and its metabolite DMG (Dimethylglycine) were determined by isocratic HPLC procedure with u.v. detection. The detection limits were specified to be 0.005 mM/l for betaine and 0.002 mM/l for DMG.

No specific pharmacokinetic animal studies have been performed. With respect to the long-standing use of betaine in humans the applicant presented human data.

No data was presented concerning tissue distribution, protein binding, placental transfer, secretion into the milk and pharmacokinetic drug interactions. Since no animal data was presented no interspecies comparisons were possible.

Safety margins are currently neither available nor considered necessary.

Toxicology

Single dose toxicity

The single dose toxicity studies were consistent with a relatively low toxicity of betaine. The main symptoms could be characterised as known symptoms of the CNS and gastrointestinal bleeding.

Published data: the acute toxicity of betaine is low. In the mouse, LD_{50} values of 10,800 mg/kg and 830 mg/kg by the subcutaneous and intravenous route respectively were reported.

Applicant's data: In the one single-dose study conducted in rats at dose levels up to 20,000 mg/kg, the combined (male and female) LD_{50} was 11,179 mg/kg. The major antemortem sign of toxicity was CNS depression. Necropsy of decedants revealed areas of body surface staining, altered stomach, small intestine and caecum contents and darkened glandular gastric mucosa and brain. Red fluid was observed in the small intestine of 2 females and in the small intestine and brain of one male at 20,000 mg/kg. Necropsy of survivors was unremarkable.
The signs of CNS depression were consistent with the anticonvulsant effects discussed in the secondary pharmacodynamics section. The delayed deaths (1/10 at 10,000 and 12,500 mg/kg) appeared to be the result of gastro-intestinal irritation and bleeding, not present in survivors and in the non-lethal doses of 5000 mg/kg.

Repeat dose toxicity (with toxicokinetics)

Studies were mainly reported from the published literature but one study was conducted with Cystadane by the applicant.

Published data: The studies conducted at BIBRA involved a dose range finding study to evaluate reversibility. The highest dose used allowed the observation of some biological effects which disappeared during the recovery period. In the definitive study betaine was added to the diet in rats at 1%, 2% and 5% (17.5 g/day) for 14 or 28 or 90 days. There was no clear evidence of toxicity but at higher betaine intakes several serum clinical chemistry parameters were altered including increased ALKP and LDH levels, decreased ALT activity, increased gamma GT and bilirubin in females and decreased albumin and protein levels in males. The Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) of the red blood cells were reduced and hepatocytes developed fatty droplets in a dose related manner. Females were more affected than males. These effects, except those on the reduced MCV and MCH, were reversed during the recovery period.

In the Brandeis University studies conducted in rats at dietary dose levels of 0.5%, 0.75%, 1 and 5% (25 g/day) for 28 days or 5% for 90 days, hepatic lipid droplets were investigated in more detail. Liver lipid was reduced by betaine. There were no significant adverse effects of clinical significance, but the

MCV was again reduced at 5% betaine in the 28 day study but all other parameters were completely reversible.

The influence of betaine on the liver and a possible imbalance in the folate-mediated metabolism was discussed. The observed effects of betaine on haematological parameters were attributed mainly to nutritional differences between the BIBRA and the Brandeis study part. The rat chow used in the Brandeis study supplied much more nutrients, vitamins and minerals than the chow used in the BIBRA part. It is reasonable that haematological effects may occur in juvenile animals which where ill-nourished especially in individuals undergoing pronounced growth. With respect to the influence of betaine on the liver it was shown that betaine enhances lipoprotein secretion and possibly apoprotein synthesis. The observed droplets likely represent VLDL particles (VLDL synthesis may be impaired due to a limited energy/protein supply in the BIBRA part), since no changes where apparent in the TGSR in the Brandeis part of the study. Again, differences in the composition of the chow may contribute to the observed influence on the liver. The data was considered satisfactory. With respect to the extensive clinical experience no evidence of adverse effects on the liver has been identified.

A 4-week study in male rats to investigate the effects of dietary betaine supplementation (0.5% w/v, approximately 1.3 g/kg) on methionine metabolism in control and ethanol-fed animals revealed that betaine had no effects on body weight gain but prevented weight loss induced by ethanol and protected against alcoholic fatty liver (Barak *et al.*, 1993).

Applicant's data: (This study has been also reported in the Pharmacodynamics Section). Liquid dietary administration of betaine at 0%, 0.5% (1.3 g/kg/day) or 1% (2.6 g/kg/day) w/v of betaine for 29 days revealed no significant treatment related effects on food consumption, body weight gain or in histopathological examination.

The repeated-dose toxicity studies do not comply with the Note for Guidance on repeated Dose Toxicity (CPMP/SWP/1042/99). However, in view of the extensive clinical experience, this was considered acceptable.

Table 2

Study ID	Species/Sex/ Number/Group	Dose/Route	Duration	NOEL/ NOAEL (mg/kg/day)	Major findings
Applicant's study. ID not given	Rat/10 sex/group	Oral, dietary Dose range 1.3 - 2.6g/kg/day	29 days	>2.6g/kg/day	None in parameters evaluated
BIBRA studies	Rat/10 sex/group	Oral, dietary Dose range 1, 2 and 5% (17.5g/day)	14 days	Not established	Clinical chemistry and red blood cell parameter changes
	Rat/40 sex/group		90 days		
	Rat/20F/group		28 days		
Brandeis studies	Rat/8 sex/group	Oral, dietary 0.5, 0.75, 1 and 5% (25g/day)	28 days	1%	Reversible decrease in MCV at 5% in 28 day study

Genotoxicity

As expected, betaine tested negative in the genotoxicity testing. Betaine was tested for mutagenicity in Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 with or without metabolic activation at concentration levels up to 5000µg /plate. This study was negative. The clastogenic potential was assessed in vitro using human lymphocytes with or without metabolic activation at concentration levels up to 10,000µg/mL for 3 hours (activated cells) or 24 hours (non activated cells). This study was negative. The clastogenic potential was assessed in vivo in a mouse micronucleus test at dose levels up to 2000mg/kg p.o. This study was negative.

In the bacterial mutagenicity assay, the relevant guideline states that the standard set of bacterial strains should include strains that will detect point mutations at A-T (adenine-thymine) sites such as S. typhimurium TA102 or E.coli WP2 uvrA. The package presented did not have these strains. However, in view of the nature of the product, the package submitted was considered acceptable.

Table 3

Type of test/study ID/GLP	Test system	Concentration range/ Metabolising system		Results
Gene mutations in bacteria M/AMES/17027	Salmonella strains TA1535, TA1537, TA1538, TA98 TA100	+/- S9	8-5000µg/plate	Negative
Chromosomal aberrations in vitro M/HL/17028	Human lymphocytes	+/- S9	1000-10,000µg/mL	Negative
Chromosomal aberrations in vivo M/MMN/17029	Mouse, micronuclei in bone marrow	+/- S9	500-2000mg/kg	Negative

Carcinogenicity

Long-term studies

There are no reports in the literature of carcinogenicity caused by treatment with betaine at doses up to 30 g daily over 6 months or 8-20 g/day for 1 to 3 years, although in view of the short duration of treatment, these findings cannot be taken as strong evidence of lack of carcinogenic potential.

As betaine is a naturally constitute of mammalian cells occurring in quite high amounts and taking into account its already long term use in clinical practice long term carcinogenicity studies were considered not necessary by the CHMP.

No data was provided concerning short or medium-term studies.

Reproduction Toxicity

No relevant studies have been conducted. The applicant stated that in view of the nature of the product and the extensive clinical experience, it was not relevant to conduct animal studies.

The applicant stated that little is known about the interactions between choline and folate and homocysteine metabolism during pregnancy despite the fact that pregnancy places considerable stress on maternal folate and choline stores and that choline is a critical nutrient for the foetus. Furthermore, the potential imbalance in folate activity and modification in purin metabolism and cell division with consequence on reproductive function during betaine supplementation were not fully explored.

The applicant reported 6 cases of pregnancy with betaine treatment without adverse effects on pregnancy or on the health of the foetus/newborn child and reported one more case which had been published in 2000 or 2003 by Gissen et al. The issue was considered acceptable by the CHMP.

Toxicokinetic data

No non-clinical data

Local tolerance

Not applicable.

Other toxicity studies

None

Ecotoxicity/environmental risk assessment

Betaine is stated to be identical to the natural constituents of mammalian tissues. After absorption, this amino acid rapidly enters the methionine/homocysteine cycle. Therefore no environmental risk can be expected from the therapeutic use of betaine. Indeed, amino acids are specifically exempted from testing in the most recent draft guideline.

Discussion on the non-clinical aspects

The effects of betaine related to the proposed therapeutic use have been investigated in several rat studies. The effects were also investigated in non-conventional species such as chick and fruit bat. The results support the use of betaine for the indication of homocystinuria.

Studies on secondary pharmacodynamics showed that betaine protected the liver against ethanol-induced injury which was related to its ability to increase hepatic SAM levels and betaine also prevented vitamin A depletion. Betaine was shown to have anti-convulsant and anti-apoptotic activity.

There are no specific studies on safety pharmacology or for pharmacodynamic reaction. It was stated that betaine occurs normally in the body, the inference being that it is unlikely to have adverse effects. After a high single dose some CNS depression signs appeared in animals (see Single dose toxicity below); it was proposed that these were consistent with the anti-convulsant effects. In view of the extensive clinical experience, the clinical data may be of greater utility in revealing potential adverse treatment related effects.

The single dose toxicity of betaine was low in rats. The CNS depressant activity only occurred at very high dose levels. This was consistent with the anti-convulsant effect of betaine reported. The irritation of the gastrointestinal tract observed is consistent with the mild gastrointestinal effects reported clinically.

Following dietary administration to rats at dose levels up to 5% of the diet for 28 or 90 days, the only adverse treatment related effects reported were small perturbations in red blood cell parameters and serum chemistry values. These were all fully reversible after 90 days recovery period. However, the information obtained for betaine from healthy animals may not be directly relevant to assessing the potential toxicity in the presence of metabolic abnormalities.

In the package of studies presented, betaine was not mutagenic. Carcinogenicity and reproduction toxicity studies were not been conducted. In view of the nature of the product, this was considered acceptable by the CHMP.