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EMA/358079/2018
Committee for Medicinal Products for Human Use (CHMP)

Withdrawal assessment report

PROHIPPUR

International non-proprietary name: sodium benzoate

Procedure No. EMEA/H/C/004150/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

AD	adverse event
API	active pharmaceutical ingredient
AR	Assessment Report
ASL	argininosuccinate lyase
ASS	argininosuccinate synthetase
CLM	metabolic clearance
CLT	total clearance
Cmax	maximum concentration
CNS	central nervous system
CoA	Certificate of Analysis
CP	carbamyl phosphate
CPS-I	carbamyl phosphate synthetase
CRF	chronic renal failure
CSF	cerebrospinal fluid
CSS	steady-state concentrations
g	Grams
GCS	glycine cleavage system
GMP	Good Manufacturing Practices
HE	hepatic encephalopathy
HV	healthy volunteers
ICH	International Council for Harmonisation
IV	intravenous
LoQ	list of questions
LPI	Lysinuric protein intolerance
MO	major objection
NAGS	N-acetylglutamate synthase
NKH	non ketotic hyperglycinemia
OTC	ornithine transcarbamylase
PA	phenylacetate
PD	pharmacodynamic
PIL	patient information leaflet
Ph. Eur.	European Pharmacopoeia
PK	pharmacokinetics
SAE	serious adverse event
SPC	summary of product characteristics
t1/2	half-life

Tmax	time of Maximum Concentration
UCD	urea cycle disorders
Vd	volume of distribution

1. Recommendation

Based on the review of the data and the applicant's response to the CHMP LoQ on quality, safety, efficacy and risk management plan, the CHMP considers that the application for Prohippur 750 mg/g granule, an orphan medicinal product, indicated as adjunctive therapy in the chronic management of non ketotic hyperglycinemia, as well as that of urea cycle disorders including carbamoyl-phosphate synthase-1 deficiency, ornithine transcarbamylase deficiency, citrullinaemia type 1, argininosuccinic aciduria, hyperargininaemia, n-acetylglutamate synthase deficiency, ornithine translocase deficiency and lysinuric protein intolerance; in all urea cycle disorders patients with neonatal-onset presentation (complete enzyme deficiencies, presenting within the first 28 days of life) and in patients with late-onset disease (partial enzyme deficiencies, presenting after the first month of life) who have a history of hyperammonaemic encephalopathy;

is not approvable since major objections still remain, which preclude a recommendation for marketing authorisation at the present time.

Questions to be posed to additional experts

None at present

Inspection issues

None at present

New active substance status

Not applicable.

2. Executive summary

2.1. Problem statement

2.1.1. Disease or condition

The proposed indications for Prohippur are as adjunctive therapy in the chronic management of non ketotic hyperglycinemia, as well as that of urea cycle disorders including carbamoyl-phosphate synthase-1 deficiency, ornithine transcarbamylase deficiency, citrullinaemia type 1, argininosuccinic aciduria, hyperargininaemia, n-acetylglutamate synthase deficiency, ornithine translocase deficiency and lysinuric protein intolerance.

It is proposed to be indicated in all urea cycle disorders patients with neonatal-onset presentation (complete enzyme deficiencies, presenting within the first 28 days of life). It is also proposed to be indicated in patients with late-onset disease (partial enzyme deficiencies, presenting after the first month of life) who have a history of hyperammonaemic encephalopathy.

2.1.2. Epidemiology

Urea Cycle Disorders

OTC deficiency which is the most frequent UCD has an incidence of 1 in 14000 to 1 in 56500 live births (Brusilow 1996, Summar 2008) and the overall incidence of UCD is 1 in 8000 to 1 in 44000 (Häberle

2012, Summar 2008). Except for OTC which is transmitted as X-linked trait, all other UCDs are transmitted as autosomal recessive traits (Brusilow 1997).

Non ketotic hyperglycinemia (NKH)

Non ketotic hyperglycinemia (NKH), also called glycine encephalopathy, is an autosomal recessive inborn error of the main glycine degradation pathway (i.e. the "glycine cleavage system" (GCS), Tada 1987) in which large quantities of glycine accumulate in all body tissues, including the central nervous system (CNS).

NKH incidence is not well-known everywhere, except in Northern Finland, where a founder effect on the P protein mutation has resulted in an incidence of 1 in 12,000 live births. Only three cases have been identified in Portugal over a period of seven years, giving an incidence of 1:47,455 (and a prevalence of 1:782,951) which compares to 1:63,000 live births in British Columbia (Verissimo 2013, Applegarth 2000).

2.1.3. Biologic features: Aetiology and pathogenesis

Urea Cycle Disorders (UCD)

Ureagenesis follows a cycle: in the mitochondrial matrix, the first step of ammonia detoxification carbamyl phosphate synthetase I (CPS-I)1 converts ammonium and bicarbonate to carbamyl phosphate (CP). The second step ornithine transcarbamylase (OTC) catalyses the condensation reaction between CP and ornithine to yield citrulline. Citrulline is transported to the cytosol where it is conjugated with aspartate to form argininosuccinic acid via argininosuccinate synthetase (ASS). Subsequently, arginine is produced by the action of argininosuccinate lyase (ASL). The final step involves the cleavage of arginine by arginase to form urea and ornithine. Ornithine must be then transported to the mitochondrion for the next turn of the cycle. In addition, another intra-mitochondrial enzyme N-acetylglutamate synthase (NAGS) catalyses the formation of N-acetylglutamate, the essential cofactor for CPS-I activity (Treem 1994). The urea cycle serves two purposes: (1) it contains, in part, the biochemical reactions required for the de novo biosynthesis and degradation of arginine, and (2) it incorporates nitrogen atoms not retained for net biosynthetic purposes into urea, which serves as a waste nitrogen product. Excess dietary protein and the nitrogenous substances produced by endogenous protein turnover are normally metabolized to yield energy and the by-product ammonia is transformed into nontoxic urea which is freely excreted in the urine.

Urea Cycle Disorder (UCD) represents a deficiency of one of six enzymes which are responsible for removing ammonia from the bloodstream by converting it into urea (e.g., carbamyl phosphate synthetase (CPS), N-acetylglutamate synthetase (NAGS), ornithine transcarbamylase (OTC), argininosuccinic acid synthetase (AS), argininosuccinate lyase (AL/ASA), arginase (AG)) (Maloney 2010). Ammonia itself is produced as an intermediate from amino acid catabolism. An accumulation of glutamine and alanine has been shown for all subtypes of UCD (Leonard 2002). Severe, prolonged and/or repeated episodes of hyperammonaemia lead to cerebral oedema and death or severe neurological impairment with mental retardation (Batshaw 1980, Msall 1984). The main treatment in UCD focuses on the alternative excretion pathway for waste nitrogen in form of ammonia by compounds that are conjugated to respective precursor amino acids (e.g., glycine) which are terminally excreted.

Non ketotic hyperglycinemia (NKH)

Non Ketotic Hyperglycinaemia (NKH) also known as glycine encephalopathy is an autosomal recessive disorder of the glycine metabolism. Patients are characterized by abnormally high glycine

concentrations accumulating in plasma and cerebrospinal fluid (CSF), which is considered to be the main cause of toxicity (Beyoglu 2012). Glycine is an important constituent of proteins, providing more than 25% of the amino acid residues of abundant structural proteins such as collagen, elastin, and gelatin. Glycine also plays an important synthetic role in metabolism of purines, glutathione, creatine, porphyrin and heme. Approximately 1 g of glycine is used daily in various conjugation reactions that play a role in several detoxification pathways (Hamosh 1995). The Glycine Cleavage System (GCS) is the major pathway for the catabolism of both glycine and serine in vertebrates (Hayasaka 1987). It is a complex of four proteins, located in the inner mitochondrial membrane of the liver, kidney, brain, and placenta. In case of faulty cleavage, glycine accumulates in all body compartments, particularly the brain, leading to severe brain damage and irreversible neurological impairment (Hoover-Fong 2004). Glycine has several physiological functions; mainly it is a biosynthetic precursor for protein synthesis, and a neurotransmitter in the CNS. Most synaptic neurotransmission in the brain involves the amino acids glutamate (excitatory), gamma amino butyric acid (GABA, inhibitory), and glycine (excitatory and inhibitory) as neurotransmitters. The symptoms of NKH relate to glycine neurotransmitter activity. Glycine acts as an inhibitory neurotransmitter at the GlyR receptor in the spinal cord and brain stem, leading to lethargy, muscular hypotonia, apnoea and hiccupping. Glycine is excitatory in the cortex at the N-methyl-D-aspartate (NMDA) receptor of glutamate, resulting in myoclonic jerks, intractable seizures and brain damage.

In both indications, NKH and UCD, sodium benzoate acts as a nitrogen scavenger, binding nitrogen in form of glycine (NKH and UCD) or ammonia (UCD), respectively, followed by urinary excretion (Arnstein 1951; Bridges 1970; Barshop 1989; Beyoglu 2012).

2.1.4. Clinical presentation, diagnosis and prognosis

Urea Cycle Disorders (UCD)

UCD may manifest at any age, even in late adulthood (Leonard 2002). However, there are certain periods when metabolic decompensations are more likely to appear and thus UCD to manifest e.g. during the neonatal period, later in infancy when breast-feeding is replaced by a formula with higher protein content or when formula is replaced by cow's milk, after consumption of high protein food, during puberty, during peripartum period, during severe diseases, infections, operations, gastroenteritis and gastrointestinal bleeding. Approximately one third to one half of patients become symptomatic in the neonatal period and another half of the patients have late-onset disease (Summar 2008).

The clinical spectrum is wide: the most severe forms occur early in life (full enzymatic deficiencies) and affect the more proximal enzymes of the urea cycle. The most severe cases have no residual enzyme activity and present with hyperammonaemic coma within the first week of life, whereas patients with milder forms have some residual enzyme activity and their clinical presentation occurs later in life with recurrent episodes of hyperammonaemia (Summar 2001).

Patients with classic neonatal form of UCD present after a short symptom-free interval of 1-5 days with poor feeding, vomiting, lethargy, muscular hypotonia, hyperventilation (respiratory failure), irritability, hypothermia, convulsions. Without rapid intervention, coma prevails as the condition worsens and leads to death. If not promptly and appropriately treated, patients die, or survive with irreversible severe brain damage (Summar 2001).

Late onset forms include signs and symptoms of cyclical vomiting, migraine-like headache, protein avoidance, lethargy, somnolence, irritability, agitation, disorientation, ataxia, psychosis, and visual impairment. Seizures can complicate the clinical picture. Delayed physical growth and mental

development are common (Batshaw 1994). Likewise, symptoms may be delayed in onset by a mild deficiency or dietary self-selection, i.e., avoidance of high-protein foods (Call 1984, Tuchman 2002).

Arginosuccinic aciduria (ASL deficiency, or ASL) is particular as it can also present with long-term complications not commonly observed in other UCD, including: liver involvement, neurocognitive deficits, trichorrhexis nodosa, hypertension and hypokalaemia (Nagamani 2012). Disabilities and neurological abnormalities were shown to be more prevalent in ASL patients with significantly higher rates of intellectual disability; a higher frequency of seizure disorders. Tone change and reflex abnormalities were reported in 25% of ASL vs. 14% of OTC patients and were significantly higher in ASL (Tuchman 2008). Subjects with ASL also had significantly increased ALT levels compared to both ASS and OTC, reflecting the greater predisposition of patients with ASL to liver disease (Tuchmann 2008).

Apart from clinical presentation, routine laboratory and analyses of amino acids in plasma and urine, and orotic acid in urine (Summar 2001), a definitive diagnosis depends on the determination of the enzyme activity from molecular genetic testing or, less frequently nowadays, from a liver biopsy specimen with enzymatic analysis. Molecular genetic testing is also used for carrier detection, and prenatal diagnosis (Häberle 2011).

Non Ketotic Hyperglycinaemia (NKH)

The diagnosis of NKH is established on an increase in plasma and CSF glycine concentrations with a glycine CSF/plasma ratio greater than 0.08 (Hamosh 1995, Applegarth 2001). Elevated glycine levels in urine, plasma, or CSF may occur in a number of other genetic disorders and clinical conditions (brain injury, hypoxic-ischemic encephalopathy, chronic renal failure), as well as by therapeutic agents (e.g. valproic acid) and technical factors (bloodstained CSF). Awareness of such influences and correct determination and interpretation of glycine levels and CSF/plasma ratio are therefore critical in avoiding false diagnosis of NKH and in justifying the decision to perform molecular and enzymatic analyses in suspected cases (Korman 2002).

Over 170 cases identified and registered with the International NKH Family Network, the largest known collection of living and deceased NKH patients in the world (Hoover-Fong 2004) indicate four presentations of the disease. Most patients present in the neonatal period with lethargy, hypotonia, hiccups, myoclonic jerks or seizures and respiratory distress syndrome or apnoea progressing rapidly to death or devastating neurological outcome (Carson 1982, Van Hove 2005). Those who regain spontaneous respiration develop intractable seizures and profound mental retardation. The prognosis is usually grim (Zammarchi 1994). However, up to 20 % of the patients presenting during neonatal period show a better mental outcome (Hoover-Fong 2004; Hennermann 2012).

An infantile presentation was described in few patients who presented with a symptom-free interval with apparent normal development for up to 6 months followed by seizures and various degrees of mental retardation.

In a third, late-onset form, patients present in childhood with progressive spastic diplegia and optic atrophy, but intellectual function is preserved and seizures have not been reported.

Finally, a variant of transient NKH has been described in 9 new-borns (Luder 1989, Schiffmann 1989, Eyskens 1992, Zammarchi 1995, Maeda 2000, Aliefendioglu 2003), who presented during neonatal period with the clinical features of NKH, a transient increase of glycine in plasma and CSF, but a good outcome with no or mainly slight neurologic sequelae. Four out of five infants had no neurologic sequelae after 6 months to 4 years of follow-up and one had a severe developmental delay at age 9 months, however most of the patients with this phenotype exhibit normal development (Aliefendioglu 2003). None of these patients had a proven enzymatic defect of the GCS and were homozygous or

compound heterozygous for mutations in the GCS genes. So far, transient glycine encephalopathy is only a clinical and biochemical phenocopy of NKH with no molecular and enzymatic bases for a defect in the GCS.

Prenatal diagnosis is possible by measuring glycine cleavage activity using chorionic villus samples. Due to the report of false negative results of these assays, DNA diagnosis is recommended for prenatal diagnosis and may be performed in those families where the specific mutation(s) is known.

2.1.5. Management

Urea Cycle Disorders

The goal of therapy in severe neonatal UCD is to provide a diet with sufficient intake of total protein, essential amino acids, and energy to promote growth and development, while preventing hyperammonaemia and hyperglutaminemia (which is by itself neurotoxic and pathogenetic in relation to ammonia levels). The goal of treatment is the same in late-onset UCD as, in these patients also, episodes of hyperammonaemia encephalopathy may result in irreversible brain damage or death (Batshaw 1986, Rowe 1986, Arn 1990). Successful therapy results in improved survival, reduced frequency of hyperammonaemia episodes, normal growth and maintenance of normal plasma ammonium and glutamine levels (Häberle 2012).

Emergency treatment of hyperammonaemia includes stopping protein intake to avoid an exogenous source of nitrogen with high caloric supplementation of carbohydrates and fat to prevent endogenous catabolism. L-Arginine (and/or L-citrulline in case of OTC) should be given immediately after the diagnosis. Essential amino acids must be gradually reintroduced as soon as circulating ammonia levels return close to the normal, within 48 hours. Combination of intravenous sodium benzoate and sodium phenylacetate removes nitrogen by alternative pathways. Haemodialysis is indicated if plasma ammonia exceeds 500 µmol/L and, in suspicion of NAGS deficiency, treatment with carbamylglutamate should be initiated (Häberle 2012).

When the ammonium level is stable at normal levels, oral medication may be gradually introduced as the intravenous medication is reduced. A couple or more days may usually be required to restore the patient to his or her specific nutritional regimen and oral medications including nitrogen scavengers.

Orthotropic liver transplantation may be considered in patients with severe forms who continue to experience episodes of hyperammonaemia despite therapy. Hepatocyte transfusion could be used until patients receive orthotropic liver transplantation. Gene therapy studies using adenovirus vectors expressing human OTC were discontinued because of severe complications (Lee 2001, Meyburg 2007).

Non ketotic hyperglycinemia (NKH)

Clinical management is based on prognosis. In practice, when the diagnosis is recognized early, the discontinuation of intensive life support measures invariably leads to rapid death. When full life support is maintained for a period (usually a week or more), subsequent survival is common but there is severe neurologic damage. There appears to be a critical period during which the infant with neonatal NKH develops enough function to remain viable, albeit grossly damaged (Luder 1989). As some patients show a better outcome it is important to look for prognostic factors which may indicate a poor/better outcome.

Unmet medical need

The applicant states there is no authorized oral presentation of sodium benzoate available in the European market. Only one registered (FDA 1987) oral formulation (Ucephan®) and one injectable formulation of benzoate (Ammonul®) are available on the US market as orphan drugs for UCD. These

products contain sodium phenylacetate sodium benzoate. These products are used for the treatment of acute hyperammonaemia and associated encephalopathy in infants, children and adults with inborn errors of the urea cycle. The applicant states that currently the off-label use of adequate dosage of sodium benzoate requires large quantities of existing formulations, which may expose the patient to potentially harmful concentrations of salts and other added compounds. Additionally, the applicant states that the off-label use of high doses of these preparations can cause side effects such as esophagitis, nausea or diarrhoea, and patient compliance is often poor due to the poor palatability of sodium benzoate. The applicant claims they have developed a pellet formulation of sodium benzoate which has no immediate taste, the active ingredient remains sodium benzoate.

There are other available therapies in the European community. Ravicti (EMEA/H/C/003822) authorised in 2015, is an oral liquid formulation of glycerol phenylbutyrate which is indicated for use as adjunctive therapy for chronic management of adult and paediatric patients ≥2 months of age with urea cycle disorders (UCDs) including deficiencies of carbamoyl phosphate-synthase-I (CPS), ornithine carbamoyltransferase (OTC), argininosuccinate synthetase (ASS), argininosuccinate lyase (ASL), arginase I (ARG) and ornithine translocase deficiency hyperornithinaemia-hyperammonaemia homocitrullinuria syndrome (HHH) who cannot be managed by dietary protein restriction and/or amino acid supplementation alone.

Pheburane, EMEA/H/C/002500, authorised 2013, sodium phenylbutyrate, 483mg/g granules, is indicated as adjunctive therapy in the chronic management of urea cycle disorders, involving deficiencies of carbamylphosphate synthetase, ornithine transcarbamylase or argininosuccinate synthetase. It is indicated in all patients with neonatal-onset presentation (complete enzyme deficiencies, presenting within the first 28 days of life). It is also indicated in patients with late-onset disease (partial enzyme deficiencies, presenting after the first month of life) who have a history of hyperammonaemic encephalopathy.

Pheburane is a hybrid of Ammonaps EMEA/H/C/000219, in which the active substance is sodium phenylbutyrate, which has been authorised in the EU since 1999. Ammonaps is indicated as adjunctive therapy in the chronic management of urea cycle disorders, involving deficiencies of carbamylphosphate synthetase, ornithine transcarbamylase or argininosuccinate synthetase.

2.2. About the product

Prohippur granules are an immediate release dosage form for oral administration, developed for use in children from the age of 6 months. Prohippur contains 750 mg sodium benzoate (ATC code A16AX11) per gram of granules as an active pharmaceutical ingredient (API). The granules have a taste-masking layer to mask the unpleasant taste of sodium benzoate. The proposed product is intended for the chronic treatment for patients with nonketotic hyperglycinaemia (NKH) or urea cycle disorders (UCD).

2.3. The development programme/compliance with CHMP guidance/scientific advice

None provided.

2.4. General comments on compliance with GMP, GLP, GCP

Satisfactory GMP certificates of compliance should be provided. The other documents relative to the GMP status were adequate.

It is not possible to confirm whether the literature studies presented were performed in accordance with the guidelines and principles of Good Clinical Practices (GCP).

No new non-clinical studies were completed. Reference is made to literature data of which compliance with Good laboratory practice (GLP) is undocumented and likely to predate implementation of GLP. Given the nature of the product and submission status as a well-established use substance, no concerns related to GLP are raised.

2.5. Type of application and other comments on the submitted dossier

This Marketing Authorisation Application for Prohippur 750mg/g Granules, has been submitted under Article 10a of Directive 2001/83/EC as amended, as a product having a well-established use within the European Community for more than 10 years with recognised efficacy and an acceptable level of safety. This is a Centralised procedure (Article 3(1) of Regulation (EC) No 726/2004) Annex (4) (Orphan designated medicinal product). Prohippur has been designated as an Orphan drug in the EU (on 14th July 2016 for carbamoyl-phosphate synthase-1 deficiency, citrullinaemia type 1, hyperargininaemia, ornithine transcarbamylase deficiency, on 29th August 2016 for lysinuric protein intolerance for ornithine translocase deficiency and on 18th November 2016 for N-acetylglutamate synthase deficiency, argininosuccinic aciduria and non-ketotic hyperglycinaemia (via transfer of designation).

3. Scientific overview and discussion

3.1. Quality aspects

3.1.1. Introduction

The finished product is presented as granules, containing the active substance sodium benzoate and the excipients hard fat, basic butylated methacrylate copolymer, colloidal hydrated silica and ethanol.

The product is contained in a HDPE container with a polypropylene child resistant cap with desiccant. Two dosing spoons (one calibrated dosing spoon is measuring 0.5 g to 5 g of sodium benzoate and the second spoon is measuring 100 mg of sodium benzoate) are proposed for the administration of the product to patients.

3.1.2. Active Substance

The ASMF procedure was used to provide the supportive documentation for the active substance.

Applicant's section regarding the active substance

The **major objection** raised at Day 120 regarding the missing information on the control of the active substance by the drug product manufacturer is **not fully resolved** at Day 180. The applicant has discussed the properties of the active substance that may impact on the quality of the finished product, e.g. polymorphism, related substances control, residual solvents, particle size, elemental impurities. However, the response is insufficient and the control of the residual solvents, the control of the particle size, the control of the elemental impurities have to be further addressed.

The specification document used by the drug product manufacturer has to be updated, CoAs have to be provided and further information is required on the analytical methods and the references substances used by the drug product manufacturer for the control of the active substance.

Applicant's part of the ASMF

The applicant provided the applicants part of the ASMF from the active substance manufacturer. In general the level of detail provided is limited, commensurate with the simplicity of the molecule sodium benzoate.

The majority of the questions initially raised on the ASMF Restricted part were not answered at Day 121. As a consequence a major objection point is raised at Day 180, to bring this to the applicant's attention.

General Information

The active substance, sodium benzoate, is widely used as an excipient in medicinal products, i.e. as an antimicrobial preservative, or as a tablet and capsule lubricant. It is described in the Ph. Eur. monograph 0123.

The applicant has provided general information- sodium benzoate is a white to off-white crystalline powder, easily soluble water and slightly soluble in alcohol. Its hygroscopicity and polymorphism have been adequately discussed. The active substance is slightly hygroscopic, and does not have any polymorphic forms.

Manufacture, characterisation and process controls

Some details of manufacture have been included. The active substance is obtained via a one-step reaction.

Characterisation by using Elemental analysis, NMR, UV, and IR has been outlined. The potential related substances were adequately investigated by forced degradation studies.

Specification

The majority of the tests included in the specification proposed for the control of the active substance are based on the Ph. Eur. monograph for sodium benzoate. Additional tests for related substances, heavy metals and residual solvents were included.

Container closure system

Details of container closure and stability have been provided. The applicant has confirmed that bags tested according to the same specification and the same packaging system have been used for stability studies (double bagged polyethylene).

Stability

Results from the stability testing programme have been provided and a 3 years retest period was proposed for the active substance. Being supported by real time stability data, this proposed re-test period is considered acceptable, if the substance is stored in the same packaging as used for stability studies, at a temperature below 25^{o}C.

3.1.3. Finished Medicinal Product

Description of the product and Pharmaceutical Development

The drug product is described as white to almost white, coated granules, containing 750 mg of sodium benzoate per gram of granules. The product is contained in a HDPE bottle, with a polypropylene child proof cap with desiccant. The granules are packaged in white HDPE bottles which are supplied with two dosing spoons: one spoon measures 0.5 g to 5 g and the second spoon measures 100 mg.

The composition is simple, consisting of the active substance, binder, film coating agent and anti-sticking agent. The excipients contained in the formulation are of Pharmacopoeial quality.

The development of the product has been described; the choice of excipients and their functions have been explained. The choice of final formulation was made on the basis of the acceptable daily intake values and the European Food Safety Authority (EFSA) opinions of the excipients, however further justification in relation to the chosen excipients is required considering the patient population. In particular, the justification for the level of the excipient basic butylated methacrylate copolymer is not considered sufficient. The quantity of basic butylated methacrylate copolymer is more than threefold the ADI limit, which is of majorn concern, and considering there is no clinical or pre-clinical data to support the quantity of basic butylated methacrylate copolymer further justification for the proposed quantity is required (**major objection**).

The development of the dissolution method was described. Additional information is required to demonstrate that the selected dissolution method conditions are justified, especially regarding the agitation speed of 150 rpm and its implications on the discriminative power of the method. Further clarification was provided regarding the studies performed to demonstrate the discriminatory power of the in-vitro dissolution method.

During the initial assessment, a clarification point was raised requesting the dissolution profile of the biobatch, in relation to a clinical question. No clinical studies have been conducted with the proposed product. The relevance of the dissolution study for batch 60554 with no *in vivo* studies conducted is limited.

A comparative dissolution study of the proposed product versus an encapsulated preparation of sodium benzoate was mentioned in the response to the clinical question 114 and a table with mean dissolution rates for the two formulations was provided. It seems that the applicant used the results from this study and PK literature data to answer the clinical question. This approach was considered not acceptable by the clinical assessors.

From a quality perspective, the data provided on dissolution is incomplete and cannot support a bridging approach. If it is wished to use a comparative dissolution study as a bridging to the bibliographic information, this should be further justified and the dissolution should be evaluated in line with the requirements in the guidance on bioequivalence (CPMP/EWP/QWP/1401/98 Rev.1). The dissolution of the two products in all the three pHs mentioned in this guideline (1.2, 4.5 and 6.8) should be evaluated, as well as in the media proposed for the quality control.

Bridging of the data will involve a critical discussion of the dissolution results, solubility of the active substance and comparison of the excipients used in the product's formulation against those in the product in the applicant's supporting literature. Attention should be paid to the any potential effect of the excipients on the absorption from the gastrointestinal tract.

Considering the lack of a bioavailability study and bridging data this issue is escalated to **a major objection**.

Further details were provided on the description of the manufacturing process. The critical steps were indicated, as well as the controls in place to ensure the product is of the desired quality.

As the granules are not suitable for administration via a nasogastric tube, which is required for administration to new-borns or to babies under the age of 6 months, a protocol for obtaining a solution from the granules was presented. A **major objection** was raised regarding the administration of the product to patients unable to swallow the product at Day 80. At Day 150 this point is **not fully resolved**, the reproducibility of this preparation among hospital pharmacies is still not fully addressed, the concern regarding the tea strainer has not been addressed and an in-vitro study demonstrating the functionality of the nasogastric tube with the drug product and the actual dose recovery to see if the process is feasible. Further concerns regarding the administration of the product to patients that are unable to swallow were expressed.

The applicant has chosen an in-vitro method to determine the palatability of the product however additional batch data is requested to show taste masking is achieved.

To support the proposed method of administration, compatibility of the granules with vanilla pudding and apple sauce was submitted however further information is required regarding the compatibility of the product with water/milk and the acceptability and palatability of the formulation in different age groups.

Manufacture of the product and process controls

The drug product manufacturing process is a standard process for the manufacture of granules. The pellets are formed by cold-extrusion and subsequently rounded by spheronisation, then coated in a fluid bed coater. The information provided for the drug product manufacturing process sections is generally acceptable, a flow diagram and narrative description of the manufacturing process and in-process controls were included.

Process validation of three production scale batches has been performed. A hold time is proposed between production of cores and coating, and between production of bulk coated granules and filling, which requires additional clarification.

Product specification

The specification proposed for the control of the drug product contains the tests required for adequately controlling the quality of the drug product. The analytical methods have been adequately described and validated. Tighter limits should be proposed for the controls of the dissolution, based on the batch results. A test of the integrity of the taste masking property was included in the release and shelf life specification.

The analytical procedures are generally well described and validated. Additional validation data is requested to demonstrate the stability indicating nature of the HPLC method used of the control of the related substances. Further validation is required for the surface spread analytical method proposed for the control of the microbiological contamination to ensure all test organisms can be detected.

Batch analysis has been performed on 3 batches. The batch analysis results show that the finished product meets the specification proposed.

Further discussion is required on the evaluation of the elemental impurities in line with ICH, which should include an assessment of the potential elemental impurities from the active substance, excipients, manufacturing equipment and container closure system.

Container closure system

Compliance with the Commission regulation (EU) No. 10/2011 is required for the primary packaging used for the storage of bulk granules. Samples of the dosing spoons are still to be provided.

Stability of the product

The stabilities studies are carried out in accordance with current ICH/CHMP guidelines and the containers used in the stability studies are the same as those proposed for routine storage. Studies were conducted at long-term (25°C / 60% RH), intermediate (30°C / 65% RH) and accelerated conditions (40°C / 75% RH). The long-term stability data cover the proposed shelf life of 24 months. Stability studies results up to 36 months were provided and compliant with the specification.

The stability data provided shows that the drug product is stable, no significant changes/variations of the product's quality are observed under long term stability conditions and intermediate stability conditions. Out of specification results were observed at accelerated conditions. The proposed shelf life of 24 months and the proposed storage conditions 'do not store above 25°C' are acceptable.

In-use stability testing was performed. This study was designed to simulate the use of the product in practice, at room temperature over a period of 2 months. The in-use stability data demonstrates the product remains within specification for an in-use shelf life of 60 days once the HDPE bottle has been opened. The results for dissolution and moisture content are close to the limits however no out of specification result occurred. The in-use stability should be regularly monitored to ensure the 60 days in use shelf life is maintained.

3.2. Non-clinical aspects

3.2.1. Pharmacology

The applicant has not performed any studies to support the proposed pharmacodynamics of sodium benzoate. Only brief summaries are provided from the literature describing both *in vitro* and *in vivo* effects of benzoate on glycine levels, as it is stated that as there is a large repository of information of the clinical use of sodium benzoate, that non-clinical data is largely superseded.

Pharmacodynamics

In vitro studies have been performed using rat kidney/liver and human liver tissue. This literature suggests the role of the benzoyl-coenzyme A (CoA) in the metabolism of sodium benzoate, the scavenging of glycine and production of hippuric acid as a result. This forms the basis of the proposed mechanism of action of sodium benzoate to treat disorders such as non-ketotic hyperglycinaemia (NKH), as well as that of urea cycle disorders (UCDs).

In vivo studies extracted from the published literature show that there is a general dose-dependent decrease in glycine levels in rats treated with intraperitoneal sodium benzoate, and a resultant increase in hippurate levels in the urine. These changes seem be of marginal effect in the main, as at a dose of 800 mg/kg sodium benzoate there is actually an increase in overall glycine concentration, so a glycine scavenging effects of sodium benzoate would appear to be limited. Other studies are reported in albino rats and rabbits, which supports the mechanism of a reduction in liver glycine concentration following treatment with intraperitoneal sodium benzoate. A single study is presented in which guinea pigs are administered oral sodium benzoate at 300 mg/kg in which there was a benzoate-dependent reduction of glycine in both the liver and in plasma. Overall in each of these studies the metabolism of sodium benzoate was predominantly observed in liver tissue.

The recommended clinical oral dosage of Prohippur is 1 g/kg/day (equal to 750 mg/kg/day sodium benzoate) in the treatment of NKH and 0.33 g/kg/day (equal to 250 mg/kg/day sodium benzoate) for treatment of UCD. In a previously reported study in Sprague Dawley (SD) rats (Beliveau 1987), the dose of 800 mg sodium benzoate/kg/day b.w, the glycine level sharply increased (123% of control), while a decrease (70% of control) was observed at 1000 mg/kg/day, the highest dose tested. The applicant has further justified the dosage recommendations in terms of clinical pharmacodynamics, and considering the well-established use of sodium benzoate, the clinical data would supersede any non-clinical findings.

No secondary pharmacodynamic data was supplied for sodium benzoate, the absence of this data has been suitably justified. Secondary pharmacodynamics related to the pharmacodynamics of major metabolite, hippuric acid, has been discussed. Hippurate inhibits glucose utilisation in striated muscles, thus contributing to muscle weakness, and has been suggested to accelerate the progression of CKDs. However, Renal tubular dysfunction has not been observed in the chronic treatment of patients with the indication at the recommended dose (UCD/NKH).

Safety pharmacology

No non-clinical discussion is supplied, as it is considered that safety concerns with respect to safety pharmacology parameters are not expected. Omission of this section in the non-clinical part is determined by existing clinical data, which does not indicate adverse effects on the cardiovascular, central nervous or respiratory system in humans. Long-term clinical experience with oral administered sodium benzoate suggests that oral intake is not expected to result in any adverse safety pharmacology effect.

No pharmacodynamic drug interactions data was supplied, which is acceptable, considering the specific pharmacodynamics of sodium benzoate.

The overall pharmacology package to support Prohippur in the treatment of patients with NKH and UCD is limited, and there is minimal non-clinical data to suggest a clear pharmacodynamic effect in the proposed indications and sub-indications, however this is discussed further in the clinical part and is addressed in more detail in the Clinical AR. From a non-clinical perspective, further discussion of the limited literature is not considered necessary.

3.2.2. Pharmacokinetics

Publications on absorption (extent and rate of absorption, *in vivo* and *in situ* studies), distribution (protein binding, blood cell transfer), metabolism (*in vitro* and *in vivo*), and excretion (routes and extent of excretion) were presented by the applicant.

A number of *in vitro* and *ex vivo* studies have demonstrated the rapid absorption of sodium benzoate/benzoic acid. This predominantly takes place in the gastrointestinal tract, and indicates high level of oral bioavailability across species. Absorption data in the non-clinical part is absent, however representative human data is presented which indicates dose dependent increase in benzoic acid concentrations and resultant increases in exposure to hippuric acid, the main metabolite for benzoic acid.

Distribution is described in a single literature article which suggests albumin binding to a limited extent of 5%, with no evidence to distribution to blood cells. Distribution has been only briefly described in both non-clinical and clinical parts and further questions are raised in the clinical part considering the extent of rapid distribution and volume of distribution. This is not pursued further from the non-clinical perspective however.

Hippuric acid or hippurate represents the main metabolite for sodium benzoate/benzoic acid, and this has been well established in animal and *in vitro* models. Sodium benzoate is converted by the benzoyl-CoA ligase in the liver into its coenzyme A (CoA) ester, benzoyl-CoA. This is then converted to hippurate following conjugation with glycine, resulting in the scavenging of glycine.

Excretion is largely via urine as hippurate. This has been investigated in a range of animal studies and been reviewed in humans additionally.

No information in the non-clinical part is described for pharmacokinetic drug interactions. Some discussion is provided in the clinical overview which amounts to two literature articles concerning the potential interaction of salicylic acid and carnitine with benzoate. Considering the importance of pharmacokinetic drug interactions, namely competition with antibiotics for renal excretion and the possible hypernatremic effect in patients with diminished renal excretion, the applicant should include this information in the SmPC **(OC)**. The applicant has referred to 2 articles from Japan which discuss the transport of uremic toxins via organic anion transporters (OAT). Little of no reference is made to these articles in the response and this is not considered a sensible approach, simple reference to literature sources with no interpretation of the findings does not adequately addressed the concerns raised in the submission. The information provided in the articles by Deguchi, *et al* should be adequately reflected in section 5.2 of the proposed SmPC **(OC)**.

There is some information provided to support theoretical interactions with penicillin, probenecid or valproic acid, this is reflected in the proposed SmPC, with the exception of an interaction with penicillin. This should be addressed **(OC)**.

The applicant has failed to address the concerns on whether hippurate is a substrate, or inhibitor, of OCT2 and Pgp **(OC)**.

3.2.3. Toxicology

General toxicity:

Acute toxicity studies are reported in the literature with sodium benzoate, although this is limited to data from a single reference. This describes effects in mice, rats, cats and rabbits following oral, dermal and inhalation exposure. Oral LD50 values ranged between 2100 – 4070 mg/kg bw in rats and the most significant effects were of diarrhoea, muscular weakness, tremors, hypoactivity, and emaciation. In cats treated with 450-890 mg/kg bw benzoic acid, there were higher indications of toxicity, 50% mortality alongside aggression, hyperaesthesia, and collapse that is attributed to the low capacity of cats for glucuronidation.

Repeat-dose toxicity has been reported in rodents, both mice and rats, in a variety of published literature studies.

In mice, animals were treated with oral sodium benzoate for up to 35 days with doses ranging from 750 to 12000 mg/kg. The most significant finding was of mortality at doses of 6000 mg/kg or higher, with signs of hypersensitivity, weight changes to the liver/kidney, changes to biochemical parameters in serum and the liver. A no observed adverse effect level NOAEL) in mice is considered to be 3000 mg/kg.

In rats, various strains have been treated for up to 90 days with oral sodium benzoate with doses ranging from 16 to 6700 mg/kg. Similar to findings observed in mice, the most prominent effect was of mortality in rats treated with high levels of benzoate, doses of 3000 mg/kg and higher. Liver and kidney weights were affected in rats treated with doses of 6290 mg/kg, and signs of intoxication included hyper-excitability, urinary incontinence, and convulsions in animals treated with 6700 mg/kg. A NOAEL of 2620 mg/kg is nominally presented in rats treated up to 90 days with sodium benzoate.

No toxicokinetic studies are presented by the applicant to support the findings from the repeat dose toxicity studies, making these observations extremely difficult to correlate with human levels of exposure. Results from repeated-dose toxicity studies suggest that the toxic effects could include changes in body weight, cases of hypersensitivity, and changes in clinical biochemistry parameters. At higher sodium benzoate doses (6000 mg/kg in mice, or ≥3700 mg/kg in rats) mortality occurs. In rodents, 4500mg/kg of sodium benzoate produced changes in the relative weight of liver (also absolute weight) and kidneys, along with changes in the lipid serum profile. The same changes were consistently observed in other studies but at lower levels (see Fujitani, 1993). Nevertheless, when considering other studies (described by Wibbertmann, 2000) it becomes very difficult to establish a clear dose threshold for toxicity. The critical analysis of the data was not performed nor provided in tabular format as requested. It's inadmissible that such critical analysis was not performed by the applicant, rendering this part of the toxicology section as practically not assessable given the low quality of the original Non-Clinical Overview submitted. No updated documents were submitted and the point remains **(OC)**.

It is acknowledged that limitations on the presented literature evidence prevent further discussions on the adequacy of the data, and there are associative concerns with effective dosing and treatment in the proposed treatment groups. Given the extent of use of sodium benzoate in the past and the clinical experience, the absence of a significant safety margin in the treatment of NKH patients may be acceptable. However there are major clinical concerns regarding the proposed posology and efficacy of Prohippur in the treatment of UCD and NKH patients that would need to be addressed.

Genotoxicity and carcinogenicity

Sodium benzoate has been extensively reviewed for its potential genotoxicity in the literature. The overall conclusion from *in vitro* and *in vivo* studies with sodium benzoate is that it is non-genotoxic.

Carcinogenicity of sodium benzoate has been reviewed in two long-term studies in mice and in rats. Mice were treated with oral sodium benzoate in drinking water during a life-long study and were dosed with 6200 mg/kg/day in male mice, and 5960 mg/kg/day in female mice. There was no evidence of tumour generation in mice at the end of the study period. Rats were treated with oral sodium benzoate for 18-24 months at doses of 500 and 1000 mg/kg/day. There was poor survival in all treatment groups in this study due to mycoplasma infections which resulted in early terminations of animals. As a result no significant conclusions were made from this study. There was however no indications of increased carcinogenic potential in rats treated with sodium benzoate from histopathological examinations of terminated animals. Overall considering the extensive clinical use of this compound and the lack of significant findings in the long-term studies in both rats and mice, it is concluded that sodium benzoate is not carcinogenic.

Reproductive toxicity

Literature data is limited in the investigation of sodium benzoate in reproductive toxicity. Reviews include effects in several benzoates: sodium benzoate; benzoic acid; benzyl alcohol; and benzyl acetate. In a study in which male rats and female pregnant rats were treated with up to 500 mg/kg/day with benzoic acid, with no observed effects on fertility or on lactation. The rats were treated over their lifetime with no effects on offspring, and findings in treated animals matching that of controls. In mice, there were no effects observed in the testes of treated animals, with no clear evidence of changes in fertility parameters. Overall there are no indications from non-clinical data to suggest an effect on fertility following dosing with sodium benzoate/benzoic acid, however it is unknown how this may reflect in humans. This should be adequately reflected in section 4.6 of the SmPC **(SmPC point)**.

Developmental toxicity has been widely reported in a number of review articles, with reference to studies completed in the 1970's. Evidence from provided from studies with rats, mice, rabbits and hamsters suggests there to be no evidence of teratogenicity, and any anomalies were only seen at doses considered to be sufficiently toxic to dams and possibly associated with reduced maternal nutrition. There is no evidence of effect from breastfeeding and whether sodium benzoate is present in breast milk. The applicant has removed contraindications for use of Prohippur in both pregnancy and breastfeeding which is reflected in the non-clinical data. A general warning for use is suggested in line with the "Guideline for risk assessment of medicinal products on human reproduction and lactation: from data to labelling" (EMEA/CHMP/203927/2005) – **(SmPC point)**.

It is also considered that the proposed population of patients are likely to be of neonate/infancy and of young age, however these patients will be treated chronically over their lifetime potentially with Prohippur and so the potential effects on use during pregnancy and during breastfeeding may be more relevant at that stage. No juvenile toxicity studies have been presented and given the extent of clinical data of the use of sodium benzoate in children the absence of this data is acceptable.

Other toxicity studies:

No concerns were raised from a range of local tolerance type studies completed with sodium benzoate. There are no data obtained from literature concerning the potential effects of sodium benzoate on antigenicity, immunotoxicity or dependence. Given the extensive clinical experience with this substance, the absence of this data is acceptable. There is however no discussions located in the non-clinical or clinical parts of this dossier.

Limits for impurities in the drug substance and drug product have been adequately justified and are in line with appropriate ICH guidelines. Residual solvents are well within tolerance limits in ICH Q3C.

A limited discussion on the relevant safety and resulting ingestion of excipients in the drug product has been provided. The applicant should further discuss the interpretation of the ICH M7 Guideline in this context for risk assessment, and discuss the lack of alternative therapies available to the patient population that would justify the high level of butylated methacrylate copolymer in the final drug product **(Combined with Quality MO).**

The applicant has failed to address concerns on the excessive levels of 'hard fat' in the formulation. The potential consequences of ingesting quantities of 'Hard fat' in patients with NKH/UCD should be discussed **(OC).**

3.2.4. Ecotoxicity / environmental risk assessment

A full environmental risk assessment (ERA) based on literature is supplied to support this application for Sodium Benzoate 750 mg/g granules.

A Phase I assessment was carried out. Logkow has been reported in literature to be -2.269, below the threshold to require further examination for persistence, bioaccumulation and toxicity (PBT). In order to determine an accurate value for PECsurfacewater, the Expert has refined penetration factor (Fpen) using prevalence data for NKH and UCD populations. The refined PECsurfacewater value however or **0.56 µg/L**, exceeds the action limit and so a Phase II ERA assessment was carried out.

The Phase II ERA assessment has been carried out with reference to literature. Absorption/desorption has been determined for sodium benzoate, it is expected to have very high mobility based upon an estimated Koc of 17, corresponding to a calculated logKoc of 1.23.

A number of literature references are quoted in the ERA document. Due to the ready biodegradability, the low bioaccumulation potential, the low toxicity for the included aquatic species and the low affinity to sludge is not expected that this medicinal product may pose a risk to the environment.

However, assuming the use of sodium benzoate as a medicinal product, it makes sense, to consider its metabolism and the main metabolite is the hippuric acid (73 to 90% excreted against <1% of benzoate). Therefore, the applicant should consider updating the ERA with adequate discussion of the potential environmental effects due to this metabolite, studies to investigate this are ongoing and this will be addressed in the next round of responses **(OC)**.

Summary of main study results

Substance (INN/Invented Name): Sodium benzoate					
CAS-number (if available): 532-32-1					
PBT screening		Result			**Conclusion**
Bioaccumulation potential- log K_{ow}	Wibbertmann 2000	-2.269			Potential PBT (N)
PBT-assessment					
Parameter	**Result relevant for conclusion**				**Conclusion**
Bioaccumulation	log K_{ow}	-			not B
	BCF	-			not B
Persistence	DT50 or ready biodegradability	-			not P
Toxicity	NOEC or CMR	-			not T
PBT-statement :	The compound is not considered as PBT nor vPvB				
Phase I					
Calculation	**Value**	**Unit**			**Conclusion**
$PEC_{surfacewater}$, default or refined (e.g. prevalence, literature)	0.56	µg/L			> 0.01 threshold (Y)
Phase II Physical-chemical properties and fate					
Study type	**Test protocol**	**Results**			**Remarks**
Adsorption-Desorption	OECD 106?	K_{oc} =17			Unknown reference
Ready Biodegradability Test	OECD 301?				Ready Biodegradable
Aerobic and Anaerobic Transformation in Aquatic Sediment systems	OECD 308	$DT_{50,\ water}$ = $DT_{50,\ sediment}$ = $DT_{50,\ whole\ system}$ = % shifting to sediment =			Not required
Phase IIa Effect studies					
Study type	**Test protocol**	**Endpoint**	**value**	**Unit**	**Remarks**
Algae, Growth Inhibition Test/*Species*	OECD 201	NOEC		µg/L	species
Daphnia sp. Reproduction Test	OECD 211	NOEC		µg/L	
Fish, Early Life Stage Toxicity Test/*Species*	OECD 210	NOEC		µg/L	species
Activated Sludge, Respiration Inhibition Test	OECD 209	EC		µg/L	
Phase IIb Studies					
Bioaccumulation	OECD 305	BCF		L/kg	%lipids:
Aerobic and anaerobic transformation in soil	OECD 307	DT50 $\%CO_2$			for all 4 soils
Soil Micro organisms: Nitrogen Transformation Test	OECD 216	%effect		mg/kg	

Terrestrial Plants, Growth Test/*Species*	OECD 208	NOEC		mg/ kg	
Earthworm, Acute Toxicity Tests	OECD 207	NOEC		mg/ kg	
Collembola, Reproduction Test	ISO 11267	NOEC		mg/ kg	
Sediment dwelling organism		NOEC		mg/ kg	species

3.2.5. Discussion on non-clinical aspects

The presented non-clinical data package is based on bibliographic information for sodium benzoate available from the public domain. No new non-clinical studies have been performed for this application, and the literature evidence considers data on both, sodium benzoate and benzoic acid, because the active moiety, benzoate, is identical.

Only brief summaries are provided from the literature describing both in vitro and in vivo pharmacodynamic effects of benzoate on glycine levels. The literature suggests the role of the benzoyl-coenzyme A (CoA) in the metabolism of sodium benzoate, the scavenging of glycine and production of hippuric acid as a result. *In vivo* studies completed in rats, rabbits and guinea pigs show that glycine levels in the liver and plasma are reduced following treatment with intraperitoneal sodium benzoate.

The recommended clinical oral dosage of Prohippur is 1 g/kg/day (equal to 750 mg/kg/day sodium benzoate) in the treatment of NKH and 0.33 g/kg/day (equal to 250 mg/kg/day sodium benzoate) for treatment of UCD. Secondary pharmacodynamics related to the pharmacodynamics of major metabolite, hippuric acid, is supplied.

Safety pharmacology for sodium benzoate are not described given the extent of clinical experience, and the lack of any secondary or safety pharmacology signals in the cardiovascular, respiratory or nervous systems.

Pharmacokinetics of sodium benzoate is only sparsely presented in both the non-clinical and clinical parts of the dossier. Absorption is stated to be rapid via the gastrointestinal tract following oral administration, with high levels of bioavailability across most species examined, including rodents and humans. Distribution data is limited to a small extent of protein binding, however metabolism and excretion of sodium benzoate/benzoic acid is widely reported in the literature. Metabolism is mainly driven in the liver, leading to the conversion of benzoate to hippurate or hippuric acid, which is mainly excreted via urine. The paucity of data to describe clinical pharmacokinetics are raised in a number of points in the LoQ, including the lack of discussion of the effect of benzoate in inhibiting or inducing drug transporters.

Toxicity has been described mainly in rodents, in rats and mice, with the main findings related to mortality at very high doses of sodium benzoate, associated with hypersensitivity, convulsions and changes in biochemical parameters in serum and the liver. These are generally addressed in the clinical part, and are reviewed as risks in the risk management plan. The applicant should revise the overview provided for the section “Repeat Dose Studies” and provide a critical analysis of the data provided in tabular format. Given the absence of proper toxicokinetic data, the discussion needs to be enriched with a comparison with the available relevant human (clinical) data. Whenever possible, an adequate NOAEL should be provided for each study.

Sodium benzoate is not genotoxic or carcinogenic. There are concerns for maternal effects, however sodium benzoate shows limited effects on fertility parameters and shows no evidence for teratogenicity. Benzoate has been extensively used in supplementation for humans, however given

the limited clinical knowledge of use during pregnancy or breastfeeding at the proposed doses, the need for caution for use is warranted, however imposing contraindications are excessive. Changes are requested for sections 4.3, 4.6 and 5.3 of the SmPC to adequately reflect this data.

Limits on impurities in the drug substance and drug product appear to be well justified, however further information is requested in terms of safety in the use of excipients in the proposed treatment populations, which is combined with the major quality objection, and the relevant risks of exposure to elemental impurities.

A full environmental risk assessment is presented, however this is considered to be incomplete and further updates are expected.

3.2.6. Conclusion on non-clinical aspects

Overall major objections are outstanding that would preclude the approval of this sodium benzoate product. Provided that these major objections and the remaining other concerns are resolved then this product may be approvable.

3.3. Clinical aspects

The application is submitted as a Centralised Procedure per the requirements of Article 10(a), well established use, of Directive 2001/83/EC thus the applicant has replaced clinical test and trial results with scientific literature.

3.3.1. Pharmacokinetics

No formal Absorption, Distribution, Metabolism, and Excretion studies have been conducted with Prohippur.

Bioavailability

Intravenous benzoate doses ranging between 4.2 and 500 mg/kg in 4 infusions were associated with mean maximal benzoate plasma concentrations ranging between 2 and 15 mmol/L within 1.5 h of infusion.

In one study (MacArthur 2004) which evaluated a combination drug of phenylacetate (PA) and sodium benzoate, two cohorts each undergoing two separate treatment periods: bolus (b), or bolus and continuous infusion (b+ civ) after either 5.5 g/m2 (N=3) or 3.75 g/m2 (N=17) were included. Peak plasma levels (2-4 mmol/L) of benzoate occurred at the end of the 1.5-h-infusion. Concentrations then declined rapidly following tmax, to the limit of detection at 6.5 and 12.5 h, respectively. Plasma hippurate was already detectable 15 min following the start of the bolus dose. The AUC of hippurate increased in proportion to the dose, whilst the AUC of benzoate increased disproportionately to the dose. The tmax of hippurate occurred earlier than that of phenylacetylglutamine (the metabolite of PA), and plateaued from 10.5 h during continuous infusion period, at approximately 200 µmol/L.

Table 4a Pharmacokinetics of infusions of benzoate in healthy volunteers

Ref. (N of subjects)	Dose	Plasma concentrations					
		Benzoic acid			Hippuric acid		
		C_{max} (µmol/L)	AUC (µmol/L x h)	t_{max} (h)	C_{max} (µmol/L)	AUC (µmol/L x h)	t_{max} (h)
Moolenaar 1978 (3)	4.2-7.0 mg/kg *(b)*	43±11	656±101	0.15	-	-	-
MacArthur 2004 (20)	3.75 g/m^2 *(b)* ~75mg/kg	2136±319	4666±859	1.5	351±65	1330±278	3.0
	3.75 g/m^2 *(b+civ)* ~150 mg/kg/d	2182±298	5949±1150	1.5	323±69	2666±640	3.4
	5.5 g/m^2 *(b)* ~119 mg/kg	3444±286	13216±3827	1.7	435±113	2912±257	0.6
	5.5 g/m^2 *(b+civ)* ~238 mg/kg/d	3746±593	20430±7255	1.5	419±120	4725±1284	5.8

(b) bolus, (civ) continuous intravenous infusion

After oral administration, single sodium benzoate doses of 40, 80, or 160 mg/kg under fasting conditions were shown to be rapidly absorbed. Measurable plasma levels occurred 30 minutes after administration. Peak concentrations of ~0.8 mmol/L and up to 2.7 mmol/L were reached after 0.5-1.8 h, increasing with the dose (Kubota 1988, Kubota 1991). There is a disproportionate increase in the AUC of benzoic acid, but not of hippuric acid, with increasing doses of sodium benzoate. The mean AUC values of benzoic acid after 80 and 160 mg/kg sodium benzoate were 3.7 and 12 times greater than after the 40 mg/kg dose, respectively.

The oral bioavailability of benzoic acid following a dose of 2 mg/kg has been correctly calculated now as 12.3%, and is limited due to high first pass extraction. The bioavailability of hippuric acid is not discussed. The absorption of benzoic acid is shown to exhibit a dis-proportional increase in exposure between doses of 40 and 160 mg/kg, hippuric acid is stated to be more linear but this appears to show a less than proportional increase in Cmax. There is no bioavailability data for the highest doses proposed for Prohippur. The SmPC states up to 750 mg/kg given 4-5 times a day with food. It is suggested that a highest single dose e.g. 160 mg/kg should be proposed which is supported by PK. **LoQ MO**

Distribution

Moolenaar 1978; Study population: 3 adult healthy volunteers aged 25-32. The relative and absolute bioavailability of various formulations (including oral) of very low doses of benzoate were evaluated in comparison to those of hippurate injected at an equivalent-benzoate dose. The applicant states that the rapid distribution phase of benzoate calculated as a tri-exponential decay is explained by its extremely rapid biotransformation to hippurate. Some larger estimates for the overall apparent volume of distribution of benzoate were found up to 80 L (vs. 30-40 L for hippurate), in accordance with its more lipophilic nature. The clearance constant for benzoate was almost equivalent to the plasma flow through the liver i.e. approximately 750 mL/min (vs. 300 mL/min for hippurate, which is cleared exclusively by renal excretion). As the metabolism predominantly takes place in the liver, a substantial first-pass effect after oral administration is expected. In contrast, the contribution of renal excretion is negligible; no free benzoate could be detected in the urine.

The volume of distribution is reported to be between 10.2 and 80.0 l following intravenous dosing and as 17.3 l following oral dosing. The latter appears inconsistent with the calculated F at low doses.

Elimination

- **Excretion**

Intravenous benzoate pharmacokinetics of doses between 4.2 and 500 mg/kg in 4 infusions is associated with mean maximal benzoate plasma concentrations ranging between 2 and 15 mmol/L within 1.5 h of infusion. Plasma clearance increases with time of infusion and with dose, indicating a saturable elimination with nonlinear pharmacokinetics. The largest portion of drug clearance was attributable to metabolic clearance and the metabolism into hippurate represents a mean ~90% of benzoate administered. Hippurate appears after a median of 1 hour, and corresponds to 70-85% higher exposures than to the parent compound.

The elimination rate of hippurate is linearly correlated with its plasma concentration. The concentration of unmetabolized benzoate in plasma after oral administration is small because of a substantial first-pass effect.

After conjugation of benzoate to glycine, the resulting metabolite (hippurate) is cleared by the kidney at fivefold the glomerular filtration rate (Batshaw 1994).

Following a low dose of 1 mg/kg, 97% was excreted as hippuric acid in urine. It is not known what this figure is for higher doses. Elimination of hippurate is proposed to be non saturable and linear up to doses of 160 mg/kg. Given the plasma profiles it might be expected that there may be some differences in the initial rate however this is not shown.

Clearance of benzoic acid decreases markedly with dose from 1 mg/kg to 250 mg/kg. It is suggested that exposure is similar in healthy volunteers and patients however data in patients is very limited. It is suggested that the PK of an oral dose of 150 mg/kg sodium benzoate in a 22 year-old man with ASSD (Oyanagi 1987, patient n°2) quite well superposes with that obtained after 160 mg/kg in a 33 year-old healthy male volunteer (Kubota 1988). Similarly, the PK obtained for 130 mg/kg in a partial OTC patient (Oyanagi 1987, patient n°1) is in-between that obtained after 80 mg/kg and after 160 mg/kg in the same healthy male volunteer. It should be noted that this is based on single subject numbers.

In a very few sick neonates (3 with UCD, 1 with sepsis) including one small for gestational age, the total clearance of benzoate was 1.00 ± 0.61 mL/kg/min (Green 1983). This mean value can be compared to that in the sole adult with UCD and PK data i.e. pt n°2 from Oyanagi (1987), applying nonlinear PK hypotheses and the value for the estimated Vmax of 90 µg/mL/h, the calculation leads to a clearance of 0.96 mL/kg/min. Moreover, in a study in 5 LPI children of 2.8-12.6 years, the estimated mean total plasma clearance range was 0.86-1.07 mL/kg/min (Simell 1986). Therefore, clearance data from Green (1983) in 3 UCD neonates are similar to the clearance values in older children or in an adult with similar conditions. However higher exposure in these patients has also been attributed to immaturity of the acylation system but it is proposed that dosing is still appropriate.

There is even more limited data for the plasma levels of hippuric acid and none at the proposed highest doses. It is proposed that the curves of hippuric/benzoic acid appear to be shifted to the right with increasing dose. In UCD patients it was shown that hippurate plasma levels were lower, a tenth those of the simultaneously corresponding benzoate levels (Simell 1986) and even after accidental benzoate overdoses the production of hippurate was not reaching toxic values (Praphanphoj 2000). A better discussion is required of hippurate levels in patients versus HV and adults versus children. **LOQ MO**

- **Metabolism**

Following oral administration, sodium benzoate is converted by acylation in the liver into its coenzyme A (CoA) ester, benzoyl-CoA. The latter compound is conjugated to glycine to form hippurate, which is excreted by the kidney. Hippurate contains 1 waste nitrogen atom, so 1 mole of nitrogen is removed for each mole of sodium benzoate administered (Batshaw 1983).

In two healthy men, a dose of 1 mg/kg (8.2 µmol/kg) of 14C-labeled benzoic acid was shown to be excreted entirely as hippuric acid: 97% of the 14C administered was excreted in the urine within 4 h of dosing and almost 100% within 12 hours (Bridges 1970). The average apparent first-order rate constant for hippurate formation is 10.5 h-1 while the average apparent first-order rate constant for hippurate excretion is 2.7 h-1 (Wu 1961). Consequently, the rate of formation of hippurate can be estimated from its urinary excretion after benzoate as from 1.5 h on, almost constant rates of hippurate excretion are observed.

- **Pharmacokinetics of metabolites**

The formation of hippurate from benzoate is extremely rapid and occurs at an almost constant rate.

The urinary excretion rate of hippurate is a function of time after benzoate (Amsel 1969). Hippurate was excreted at a constant rate of ~2.1 g/h (~12 mmol/h or 0.13 mmol/kg/h) from ~1 h to ~3 h after benzoate administration. Moreover, when benzoate was administered together with glycine, hippurate excretion rate doubled to ~4.1 g/h (23 mmol/h or 0.25 mmol/kg/h). The renal excretion of hippurate is not rate limited by the capacity of the renal tubular transport system even at the highest excretion rates obtained after administration of benzoate but by the availability of glycine. Consistent with this, are both the increase with increasing dose in the fraction of benzoate excreted as a benzoyl glucuronide (from a mean 1.6% to 3.3% after 2 g and 5 g benzoate, respectively), and the decrease in this latter fraction when glycine is administered together with benzoate (from 3.3% to 0.6% after 5 g benzoate without and with glycine, respectively).

Kubota (1988 and 1991) confirmed earlier findings that the cumulative urinary excretion of hippuric acid reached a plateau 6 h after the 40 and 80 mg/kg doses (12 h after 160 mg/kg). The mean cumulative amounts of hippuric acid excreted in urine were 83%, 90% and 73% of the administered doses of 40, 80 and 160 mg/kg sodium benzoate, respectively ($p<0.05$). The mean renal clearance tended to be less after 160 mg/kg than after the lower doses.

Dose proportionality

Slopes of post-absorption concentration-time curves increased with dose, with mean values of 289, 620 and 694 µmol/L.h-1 after 40, 80 and 160 mg/kg benzoate, respectively. This translated into a disproportionate increase in the AUC of benzoic acid, but not of hippuric acid, with increasing doses of sodium benzoate. The corresponding mean AUC values of benzoic acid after 80 and 160 mg/kg sodium benzoate were 3.7 and 12 times greater than after the 40 mg/kg dose, respectively. This indicates that the biotransformation of benzoic acid to hippuric acid follows a saturable, non-linear, Michaelis-Menten's kinetics in man.

Table 5 Pharmacokinetics of oral sodium benzoate in healthy volunteers

Ref. (N of subjects)	Dose (mg/kg)	Benzoic acid			Hippuric acid		
		C_{max} (µmol/L)	AUC (µmol/L x h)	t_{max} (h)	C_{max} (µmol/L)	AUC (µmol/L x h)	t_{max} (h)
Moolenaar 1978 (7)	4.2-7.0	43±11	656±101	0.15	173±29	5821±978	0.17
Kubota 1988 (1)	40	826	761	0.5	262	436	1
	80	1307	2718	1.5	245	781	2
	160	2275	8815	2	327	1959	4.5
Kubota 1991 (6)	40	817±46	856±61	0.5	173±8	300±13	1.3
	80	1662±110	3159±132	0.8	196±13	611±42	2.1
	160	2758±232	10303±766	1.8	206±11	1249±68	4.1

Inter-individual variability as well as the influence of other factors (dietary glycine intake, availability of acetyl Co-A and/or L-carnitine which are required for the biotransformation of benzoic acid to hippuric acid) in the maximum rate of metabolism (17.2 - 28.8 mg/kg.h-1) an increase in the daily dose of sodium benzoate could result in a dose-disproportionate increase in plasma concentrations in some patients.

The applicant states that the determination of the urinary excretion of hippuric acid after the application of an oral dose of sodium benzoate would help to estimate the maximum rate of the metabolism of benzoate to hippuric acid; and the maximal rate of excretion of hippurate can be interpreted as the maximum rate of benzoate metabolism. A dose of sodium benzoate less than the maximum urinary excretion rate of hippuric acid should prevent the accumulation of benzoic acid. The applicant also states that benzoate plasma concentrations can also be directly determined and this may give much more information on the inter-individual benzoate metabolism.

Intra- and inter-individual variability

In the Kabuto paper variability appears to be moderate however in neonates it is reported to be high (Green 1983) (see below). The applicant does refer to the fact that inter individual variability due to liver enzyme maturity may influence biotransformation of benzoic acid to hippuric acid and thus impact the risk of toxicity due to benzoic acid. In addition, intra individual factors such as dietary glycine intake, availability of acetyl Co-A and/or L-carnitine which are required for the biotransformation of benzoic acid to hippuric acid are stated as leading to high variability.

Pharmacokinetics in target population

Balance studies were conducted in 3 male children with neonatal OTC under benzoate IV therapy (500 mg/kg/day in 4 infusions) in comparison to new-borns (including 1 OTC) not receiving benzoate (Green 1983).

Table 4b Benzoate balance in UCD newborns

Patient	Period of treatment (d)	Weight (kg)	Balance (mmol over 24 h)			
			In	*Out*		
			benzoate	benzoate	hippurate	Total
1	3-240†	3.35	13.2	7.5	8.6	16.1
3	3-5†	2.00	12.6	10.5	1.4	11.9
4	3-7†	3.37	13.1	1.6	7.6	9.2
Mean (SD)		2.9±0.8	13.0±0.3	6.5±4.5	5.9±3.9	12.4±3.5
RDS controls (n=7)		-	0	0.03±0.05	0.14±0.1	-
OTC control (n=1)		4.00	0	0	0.31	0.31

RDS: respiratory disease syndrome; †death; pat. n°2 not a UCD patient: hyperammonemia due to sepsis (removed from calculations above)

Two studies highlight the ability of infants with UCD/LPI to convert benzoate to hippurate and excrete the latter compound in the urines, which demonstrates the potential excretion of nitrogen via this alternative pathway (Green 1983, Simell 1986).

Table 4d Mean (range) pharmacokinetics parameters of benzoate and hippurate in UCD/LPI patients

Ref. (patients)	Dose	Benzoate				Hippurate		
		C_{max} mmol/L	t_{max} (h)	C_{ss} mmol/L	$t_{1/2}$ (h)	C_{max} mmol/L	C_{ss} mmol/L	t_{max} (h)
Green 1983 (3 UCD)	3.5 mmol/kg/d in 4 infusions	-	-	7.0 2.14-16.0	3.2 0.75-7.4	-	0.86 0.56-1.3	-
Simell 1986 (6 LPI)	2 mmol/kg single dose	6.01 5.17-6.98	2	-	4.55	0.24 0.14-0.4	-	2

Pharmacokinetic studies highlight relatively large variations in the half-life, total (CLT), and metabolic (CLM) clearances of benzoate, a single outsider (subject n°3) with considerably long half-life and low clearance rates explaining these findings (Green 1983). This five-fold difference in CLM, in drug half-life and in steady-state benzoate concentrations (Css) were possibly related to an impaired renal function with arterial hypotension.

Table 4c Pharmacokinetics parameters of benzoate and hippurate in UCD newborns

Patient	Benzoate					Hippurate	
	C_{ss} (mmol/L)	V_d (L/kg)	$t_{1/2}$ (h)	CL_M (mL/kg/min)	CL_T (mL/kg/min)	C_{ss} (mmol/L)	CL_u (mL/kg/min)
1	2.14	0.087	0.75	1.46	1.47	0.56	0.94
3	16.0	0.142	7.4	0.05	0.33	1.3	0.0
4	2.96	0.086	1.5	0.54	0.63	0.73	1.84
Mean (SD)	7.0±7.8	0.10±0.03	3.2±3.6	0.68±0.7	0.8±0.6	0.86±0.4	0.93±0.92

SS steady-state; pat. n°2 not UCD patient: hyperammonemia due to sepsis (removed from calculations)

Single doses: Pharmacokinetics after oral administration of sodium benzoate in one ten-year-old OTC girl treated with 130 mg/kg/d and one ASS man treated with 150 mg/kg/d showed plasma peaks of benzoate ranging between 1.6 and 3.3 mmol/L within 1-2 h after administration (Oyanagi 1987). The decrease in serum benzoate followed linear elimination kinetics. The short half-life (t1/2) suggests fast metabolism (mainly conjugation with glycine) and rapid distribution into tissues.

Table 7 Pharmacokinetics parameters of benzoate in UCD patients

Patient	V_{max} (µmol/L/h)	$t_{1/2}$ (h)	C_{ss} expected for 250 mg/kg IV (1.7 mmol/kg)	C_{ss} expected for 500 mg/kg IV (3.5 mmol/kg)
1	1246	1.3	385	2704
2	738	2.8	598	Inf.
Mean (SD)	991±359	2.0±1.0	491±150	NA

The short half-life (t1/2) also reported by others in LPI patients (Simell 1986) suggests fast metabolism (mainly conjugation with glycine) and rapid distribution into tissues.

Multiple doses: pharmacokinetic studies in one late-onset OTC boy treated with sodium benzoate (375 mg or 2.6 mmol/kg/d; Feillet 1998) and in one patient with classical NKH treated with sodium benzoate (750 mg or 5.2 mmol/kg/d; Van Hove 2005) confirm that sodium benzoate is rapidly converted to hippurate which itself is cleared more slowly. Repeated doses of benzoate resulted in plasma concentrations increasing during the day and returning to lower values, or even to baseline, between administrations.

Special populations

- **Impaired renal function**

The pharmacokinetics of benzoate was studied in patients with renal impairment (Mitch1982). Following benzoate administration, there was a synthesis of 4.86 g hippurate nitrogen and daily hippurate nitrogen excretion increased by 0.68 ± 0.05 g of nitrogen, representing 70 % (53%-95%) of the benzoate administered, an output close to that found in healthy volunteers (Kubota 1988, Kubota 1991). The additional hippurate was subsequently excreted and therefore all benzoate was converted to hippurate. There was no increase in plasma benzoate concentration on the morning after benzoate was discontinued, indicating that benzoate does not accumulate in CRF patients. Despite the excretion of large amounts of glycine as hippurate, there was no significant change in plasma concentrations of glycine or serine. This suggests that nitrogenous precursors of urea were used for synthesis of glycine and subsequently hippurate, leading to a reduction in net urea production.

Mean (range) excretion constant for exogenous hippurate (KE) was shown to be lower in 15 patients with chronic renal failure with values of 1.2 h-1 (0-2.1) vs. that in healthy subjects (Wu 1961).

- **Impaired hepatic function**

Only 9 patients with liver disease have been studied (Wu 1961). Intravenous administration of 2 mmol/L sodium benzoate or sodium hippurate gave normal (2.4-3.2 h-1) excretion constants for exogenous hippurate. The values for the hippurate synthesis constant ranged from 8.1 to 15.4 h-1, i.e. they were comparable to values in healthy control subjects. The conversion to hippurate was relatively slower which suggests that in patients with liver disease the capacity of the metabolic pathway for benzoate could be slightly reduced.

Considering the paucity of information regarding renal and hepatic impairment, the applicant should review the data and justify the currents statements made in the product literature. **LoQ**

Interactions

No specific studies were conducted by the applicant.

The applicant has presented one literature report demonstrating that there was no apparent effect on the elimination of hippurate from the administration of 2-3 g of salicylic acid 2h before benzoic acid.

Salicylate had no measurable effect on the proportion of hippuric acid and benzoyl glucuronide excreted in the urine after benzoate administration, and on the excretion rate constant of hippuric acid (Amsel 1969). Conversely excretion of salicyluric acid which had reached a plateau at about 1 h was immediately decreased after benzoate administration, and increased again to return to the plateau level in ~5 h. Benzoate apparently had a marked, rapid, short dose-dependent inhibitory effect on salicylurate formation.

Benzoate apparently interferes with carnitine and may promote carnitine deficiency (through the elimination of benzoylcarnitine. Supplementation with carnitine may be necessary to avoid induction of carnitine deficiency during benzoate therapy (Hennermann 2012).

Exposure relevant for safety evaluation

When plasma ammonia exceeds 250 µmol/L, the response being stoichiometric, the efficacy of benzoate is limited by potential toxicity (Green 1983, Batshaw 2001). The provided information is not sufficient to inform on the exposure of benzoic acid and hippuric acid in patients at the proposed dose. It appears that the data in patients is too limited however a better summary of the data e.g. in a mechanistic population model may assist in the understanding, alternatively more clinical PK data will be required in patients **MO LoQ.**

3.3.2. Pharmacodynamics

No studies have been conducted by the applicant.

(Green 1983) evaluated the pharmacodynamic effect of benzoate on ammonia plasma levels in 3 children with neonatal OTC undergoing peritoneal dialysis and/or haemodialysis and receiving benzoate IV therapy (500 mg/kg/day in 4 infusions) in comparison to 1 OTC new-born not receiving benzoate. (Simell 1986) evaluated this effect after alanine-induced hyperammonaemia in 5 LPI children. The studies indicated that the infants could convert benzoate to hippurate and excrete hippurate which suggest that UCD/LPI infants can use this alternative pathway to excrete nitrogen, thereby facilitating clearance of waste nitrogen excess. It should however be noted that the new-born children in the study conducted by Green were also on peritoneal and/or haemodialysis, which might explain in part the rapid decrease of ammonia levels, it is possible that sodium benzoate also contributed to the reduction of ammonia levels from a mean of ~2600 µmol/L to a mean of ~200 µmol/L (Green 1983).

(Batshaw 1983) evaluated 9 UCD patients aged 12-46 months on chronic treatment with oral benzoate (250 mg/kg/d) together with arginine (or citrulline 200-700 mg/kg/d) and a low protein diet (0.5-0.7 g/kg/d). This was found to be effective in maintaining plasma ammonium within normal limits at a mean (±SE) level of 36±2 µmol/L (Batshaw 1983).

Table 8 Benzoate effect on NH_3 in UCD or LPI patients

Ref.	Patient (diagnosis)	Age of treatment	Weight (kg)	Sodium benzoate		NH_3 (µmol/L)	
				Route	Dose (mg/kg/d)	Before (or ctrl)	After
Green 1983	1 (OTC)	3-240 d †	3.35	IV	500	4045	30
	3 (OTC)	3-5 d †	2.00			2250	170
	4 (OTC)	3-7 d †	3.37			1600	445
	Mean (SD)		2.9±0.8			2632±1266	215±211
	OTC control (1)		4.00	-	-	2100	<100
Batshaw 1983	N=9 (UCD)	12-46 months	NS	PO	250	-	36±2
	N=1 (UCD)	Not specified			800*	40	16
Simell 1986	N=5 (LPI)	2.8-12.6 y	-1.6SD	IV alanine load	288	28-411	17-213 (nss)

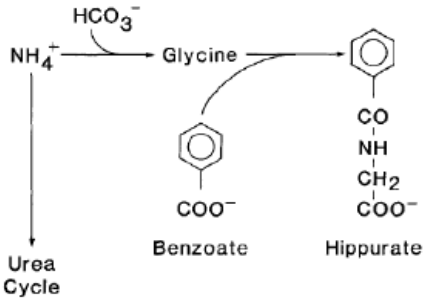
† Died; * Inadvertent overdose; NS Not specified; nss not statistically significant; SD standard deviation; in Green 1983, OTC control evolution following hemodialysis described in Wiegand 1980 and other OTC on peritoneal and/or hemodialysis; in Batshaw 1983: UCD = CPS, OTC&ASS

Mechanism of action

No studies have been conducted by the applicant.

Following oral administration, sodium benzoate is converted by acylation in the liver into its coenzyme A (CoA) ester, benzoyl-CoA. The latter compound is conjugated to glycine to form hippurate, which is excreted by the kidney. Hippurate contains 1 waste nitrogen atom, so 1 mole of nitrogen is removed for each mole of sodium benzoate administered (Batshaw 1983). In the presence of large amounts of benzoate and glycine, the level of free CoA is a rate limiting factor in the formation of benzoyl-CoA, itself a rate-limiting factor for glycine-N-acylase (Gregus 1992). The increased conjugation of glycine with benzoate tends to concomitantly reduce the glycine pool (Van Hove 1995). However, there was no obvious effect on the plasma glycine concentrations which, in the UCD population, remained within normal limits during benzoate treatment.

Figure 1: Detoxification of NH_3 by benzoate-hippurate pathway



metabolite- hippurate.

Primary pharmacology

Benzoate is rapidly metabolized after conjugation with hippurate which removes significant amounts of nitrogen (Oynagi 1987, Kubota 1988, Kubota 1991). The relative nitrogen excretion as hippurate after alanine+benzoate accounted for ~12% of that in urea in the first 6 h (Simell 1986) and can reach up to 30% (Tremblay 1993, Bachmann 2005).

Table 10 Conversion of benzoate to hippurate in healthy volunteers and in UCD patients

Ref.	Type (N of subjects)	Age	Benzoate (mmol/d)	Hippurate (mmol/d)	Ratio* mean±SD and/or range
Snapper 1946	Adult HV (9)	Not Specified	40.0	37.9±3.2	0.9±0.1 0.81-1.05
Amsel 1969	Adult HV (3)	24-34 y	0.15-0.38/kg	0.15-0.29/kg	0.94-1.05
Bridges 1970	Adult HV (2)	Not Specified	0.6	Not specified	0.994-0.997
Kubota 1988	Adult HV (1)	33 y	0.27-1.1/kg	0.16-0.89/kg	0.58-0.89
Kubota 1991	Adult HV (6)	22-34 y	0.27-1.1/kg	0.25-0.95/kg	0.82±0.04 0.73-0.9
Green 1983	UCD (3)	3 days, 3 days, 7 months	13.0±0.3	5.9±3.9	0.45±0.29 0.11-0.65
Batshaw 1983	UCD (8)	12-46 months	1.73/kg	Not specified	Mean not specified 0.2-0.37
Brusilow 1984	CPS (2)	5; 12 months	1.7-3.5/kg	Not specified	0.18-0.57
Barshop 1989	UCD (2)	1 d & 7 months; 1 month	1.25-4.5/kg	0.52-1.84/kg	Mean not specified 0.12-1.47
Feoli-Fonseca 1996	UCD group II (7)	6.2-14.9 y	49.7±13.9	19.5±13.5	0.4±0.24 0.2-0.88
	UCD group III (4)	5.1-23.5 y	33.7±9.8	22.5±14.0	0.62±0.25 0.29-0.84

(*) hippurate/benzoate ratio

Sodium benzoate effectively lowered the plasma concentrations of glycine in NKH patients. The level of glycine was reduced by a factor of two, decreasing from a mean (±SE) of 1070±150 µmol/L before treatment with benzoate to 560±150 µmol/L at a benzoate dose of 3-3.5 mmol/kg per day (430-500 mg/kg per day).

Relationship between plasma concentration and effect

In both groups of patients (UCD and NKH) there was a positive linear correlation between benzoate dose and hippurate excretion (Barshop 1989). In the patients with NKH the percentage of benzoate excreted as hippurate (~75%) was significantly higher ($p<0.01$) than that (~41%) in the patients with UCD. This study confirms the observation that sodium benzoate is converted to and excreted by at least 75% as hippurate in NKH patients, where the dose of benzoate was limited by its safety.

Conversely in patients with UCD, the capacity of benzoate was limited by relative hippurate synthesis (Barshop 1989). If hippurate synthesis is not efficient enough, unchanged benzoate plasma levels may increase. Therefore, in the neonatal period in UCD where induction of hippurate synthesis may be delayed because of an immature N-acetylase (Mawal 1997), plasma benzoate concentrations may reach potentially toxic levels and should be monitored to avoid any toxicity (Feillet 1998). It is also recommended to determine benzoate plasma levels in all patients on a high benzoate dose. **LoQ**

If the renal function is normal, then the liver glycine content, an impaired absorption or an increased glucuronidation of benzoate may be responsible for the inconstant level of hippurate production/elimination in UCD patients (Feoli-Fonseca 1996). Conversely, out of eight episodes of hyperammonemia treated with a single dose of intravenous or oral sodium benzoate (250 mg/kg), in the only UCD patient (OTC, 11 months old male subject) which did not respond to benzoate (plasma ammonium before/after values remaining flat around ~200 µmol/L, at 16 months of age), the plasma glycine level was low at 92 µmol/L (normal 122-338 µmol/L; Batshaw 1980, Brusilow 1980). During a previous episode (at 14 months of age), at a time his glycine level was normal at 332 µmol/L, treatment with benzoate resulted in a fall in plasma ammonium concentration from 207 to 55 µmol/L.

Thus, glycine availability may well be rate limiting for hippurate synthesis in UCD patients, thereby accounting for the lack of effect on plasma ammonia level in some instances.

When plasma ammonia exceeds 250 µmol/L, the response being stoichiometric, the efficacy of benzoate is limited by potential toxicity.

The pharmacodynamic effects of single oral doses of 180-650 mg/kg/d (1.1-4.5 mmol/kg/d) sodium benzoate on the plasma concentrations of glycine, benzoate and hippurate were evaluated in 2 patients with UCD (1 male OTC aged one month, 1 female ASS newborn) and in 7 patients with NKH (2 males, 5 females; 3 days-3 years, average 14.6 months; Barshop 1989). Sodium benzoate effectively lowered the plasma concentrations of glycine in NKH patients (Figure 15). The level of glycine was reduced by a factor of two, decreasing from a mean (±SE) of 1070±150 µmol/L before treatment with benzoate to 560±150 µmol/L at a benzoate dose of 3-3.5 mmol/kg per day (430-500 mg/kg per day). An effect of similar magnitude was found in a clinical follow-up study (Hamosh 1998) where 4 NKH patients received oral sodium benzoate at daily doses ranging between 500 and 750 mg/kg/d (3.5-5.2 mmol/kg/d) in multiple (6) administrations (Hamosh 1992). Benzoate was effective in reducing plasma glycine concentration by a mean factor of ~2, frequently to normal ranges. Moreover, CSF glycine concentration was also reduced by a mean factor of ~2, though never to normal ranges. Glycine concentrations in plasma (and in CSF) are age dependent (higher in new-borns) and the goal of treatment with benzoate in NKH patients are to reduce glycine plasma concentrations < 250 µmol/L).

Table 9 Benzoate effect on plasma and CSF glycine concentrations in NKH newborns

Ref.	Pat.	Benzoate treatment		Glycine (µmol/L)			
				Plasma		CSF	
		Period	Dose (mmol/kg/d)	Before	After	Before	After
Barshop 1989	N=5	3d-42months	1.1-4.5 single dose	1070±150	560±150 (after 1wk)	-	-
Hamosh 1998	1	2d-5y	3.5-5.2 in 6 times	689-943	81-946	150	27-81
	2	Birth-34months	3.5-5.2 in 6 times	475	170-531	80	62-80
	3	Birth-27months	3.5 in 6 times	857	117-640	87	36-67
	4	2d-12weeks†	3.5-5.2 in 6 times	807	83-533	135	19-91
	N=4	mean±SEM	3.5-5.2	707.0±98.1	329.1±93.4	113.0±20.0	59.4±12.7

3.3.3. Discussion on clinical pharmacology

No formal Absorption, Distribution, Metabolism, and Excretion studies have been conducted with Prohippur. The provided published literature reports regarding pharmacokinetics are not well presented and are somewhat limited as they fail to address several areas altogether and provide only limited data for others.

There is literature data for low intravenous doses which suggests oral bioavailability is 12.331% for benzoate and 92% for hippurate, there is also other data at higher doses following infusions, however this needs to be better presented. Published studies have also demonstrated that following oral administration of sodium benzoate (40-160mg/kg) under fasting conditions in adults (n=6) absorption occurs rapidly, with measurable plasma levels within 30 minutes. Tmax for sodium benzoate ranged from 0.5 to 1.8 hours and for its metabolite hippuric acid was 1-4.5 hours. One study in the literature provides some information to support a lack of an effect of food on hippurate elimination. The timing of administration of benzoate (and appearance of hippurate) relative to meal intake is aimed to

correspond to rising levels of nitrogen produced from dietary amino acids' absorption. The oral bioavailability of benzoic acid following a dose of 2 mg/kg has been correctly calculated now as 12.3%, and is limited due to high first pass extraction. The bioavailability of hippuric acid is not discussed. The absorption of benzoic acid is shown to exhibit a dis-proportional increase in exposure between doses of 40 and 160 mg/kg, hippuric acid is stated to be more linear but this appears to show a less than proportional increase in Cmax. There is no bioavailability data for the highest doses proposed for Prohippur. The SmPC states up to 750 mg/kg given 4-5 times a day with food. It is suggested that a highest single dose e.g. 160 mg/kg should be proposed which is supported by PK.

The volume of distribution is reported to be between 10.2 and 80.0 l following intravenous dosing and as 17.3 l following oral dosing. The latter appears inconsistent with the calculated F at low doses.

Following a low dose of 1 mg/kg, 97% was excreted as hippuric acid in urine. It is not known what this figure is for higher doses. Elimination of hippurate is proposed to be non saturable and linear up to doses of 160 mg/kg. Given the plasma profiles it might be expected that there may be some differences in the initial rate however this is not shown.

Clearance of benzoic acid decreases markedly with dose from 1 mg/kg to 250 mg/kg. It is suggested that exposure is similar in healthy volunteers and patients however data in patients is very limited. It is suggested that the PK of an oral dose of 150 mg/kg sodium benzoate in a 22 year-old man with ASSD (Oyanagi 1987, patient n°2) quite well superposes with that obtained after 160 mg/kg in a 33 year-old healthy male volunteer (Kubota 1988). Similarly, the PK obtained for 130 mg/kg in a partial OTC patient (Oyanagi 1987, patient n°1) is in-between that obtained after 80 mg/kg and after 160 mg/kg in the same healthy male volunteer. It should be noted that this is based on single subject numbers.

In a very few sick neonates (3 with UCD, 1 with sepsis) including one small for gestational age, the total clearance of benzoate was 1.00 ± 0.61 mL/kg/min (Green 1983). This mean value can be compared to that in the sole adult with UCD and PK data i.e. pt n°2 from Oyanagi (1987), applying nonlinear PK hypotheses and the value for the estimated Vmax of 90 µg/mL/h, the calculation leads to a clearance of 0.96 mL/kg/min (cf. Questions 124 and 129). Moreover, in a study in 5 LPI children of 2.8-12.6 years, the estimated mean total plasma clearance range was 0.86-1.07 mL/kg/min (Simell 1986). Therefore, clearance data from Green (1983) in 3 UCD neonates are similar to the clearance values in older children or in an adult with similar conditions. However higher exposure in these patients has also been attributed to immaturity of the acylation system but it is proposed that dosing is still appropriate.

There is even more limited data for the plasma levels of hippuric acid and none at the proposed highest doses. It is proposed that the curves of hippuric/benzoic acid appear to be shifted to the right with increasing dose. In UCD patients it was shown that hippurate plasma levels were lower, a tenth those of the simultaneously corresponding benzoate levels (Simell 1986) and even after accidental benzoate overdoses the production of hippurate was not reaching toxic values (Praphanphoj 2000). A better discussion is required of hippurate levels in patients versus HV and adults versus children.

This information is not sufficient to inform on the exposure of benzoic acid and hippuric acid in patients at the proposed dose. It appears that the data in patients is too limited however a better summary of the data e.g. in a mechanistic population model may assist in the understanding, alternatively more clinical PK data will be required in patients **LoQ MO.**

The applicant states that measurement of sodium benzoate plasma levels should be made, due to the risk of dose-disproportionate increase in plasma concentrations in some patients, to prevent accumulation of benzoic acid and thus maintain a positive risk benefit profile. The submitted SPC also states that benzoate levels and plasma glycine should be measured in patients with NKH prior to

dosage change. Furthermore, the SPC states in Therapeutic monitoring that “Depending on the indication, plasma levels of serine, glycine or ammonia, arginine, essential amino acids, carnitine and serum proteins should be maintained within normal limits. Plasma glutamine should be maintained at levels less than 1,000 µmol/L.” The applicant should provide evidence that assays for these analytes are currently available to clinical metabolic centres and fulfil the validation criteria for clinical use.

The applicant claims that the maximum benzoate level in patients with UCD occurred within 3 to 5 hours of dosing, and plasma benzoate levels ≥ 3.0 mmol/L (366 mg/L benzoic acid plasma concentration) should be avoided in UCD patients. It is noted that in the SmPC there is a requirement to monitor benzoate plasma concentrations in NKH patients but not UCD patients and the reason for this is not clear. On the other hand, in the guideline (Häberle 2012) the following statement is included: “Serum/plasma levels of benzoate/PBA and plasma levels of arginine (aim are fasting levels of 70–120 µmol/L) should be monitored to adjust dosages and in case of high or repeated doses and “sodium benzoate is toxic only at plasma levels >2 mmol/L”. As originally requested the applicant should clarify if the SmPC should be updated with the recommendation for monitoring of the benzoate plasma concentrations in UCD patients. The relevant SmPC wording should be proposed (information on the timeframe of this monitoring and acceptable plasma benzoate levels should be provided together with the relevant justification).

In relation to NKH patients the following recommendation is included in the SmPc: To prevent toxicity, the dose of benzoate should then be gradually increased, e.g. by 50 mg/kg/d, and plasma glycine and benzoate levels should be monitored before any subsequent dose change. This recommendation is not clear. The applicant should clarify in what benzoate serum concentration the dose could be increased. The relevant SmPC wording should be proposed by the applicant together with the relevant justification. The applicant claims that plasma benzoate levels should remain < 5 mmol/L in NKH patients however this concentration is higher than the concentration that is considered safe for UCD patients. The applicant has presented literature reports regarding the use of a glycine index to grade the severity of the NKH and base posology on. The applicant should further discuss whether the use of the glycine index is routinely clinically used in this manner in this patient population. The applicant should justify the use of glycine index in this manner and update the product literature with the relevant information **LoQ.**

There is very limited presented information regarding the impact of hepatic or renal impairment on the PK of sodium benzoate. The applicant states in the SPC section 4.5 that “benzoate does not accumulate in patients with chronic renal failure.” This statement however appears to be based on limited data and other studies have shown that hippurate excretion is reduced with renal impairment suggested an accumulation of benzoate. The information provided to health care professional regarding the use of sodium benzoate in impaired renal function is thus confusing as section 4.2 of the SPC states that “Since the metabolism and excretion of sodium benzoate involves the liver and kidneys, Prohippur should be used with caution in patients with hepatic or renal insufficiency.” The applicant discussed a few studies where benzoate was given to patients with different stages of renal failure. The applicant did not provide interpretation of these data. No discussion was provided regarding the need for the product to be contraindicated in patients with severe renal failure. It has been highlighted that in subjects with severe renal impairment a marked depression in benzoate return was observed, i.e. the mean percentage of benzoate transformed and eliminated as hippuric acid was decreased to 41.9% vs. 92% observed in normal subjects. In addition excretion constant for exogenous hippurate was shown to be lower in patients with chronic renal failure as compared to healthy subjects. No discussion on the clinical implications of these findings were provided. No discussion was provided for patients on dialysis. Further discussion and interpretation of the provided data is required. No discussion was provided for patients on dialysis. No discussion was provided regarding the need for the product to be contraindicated in patients with severe renal failure. In the SmPC it needs to be highlighted that only

limited information is available on the metabolism and excretion of sodium benzoate in patients with impaired renal function **LoQ.**

The applicant discussed two studies where sodium benzoate or sodium hippurate was given to subjects with hepatic impairment. It is noted that both studies are very old (1961 and 1925). Both studies indicating that in patients with disease the capacity of the metabolic pathway for benzoate could be slightly reduced. It is noted that benzoate has been used in exploratory studies to treat hepatic encephalopathy in subjects with cirrhosis. In the SmPC it should be highlighted that only limited information is available on the metabolism and excretion of sodium benzoate in patients with impaired hepatic function **LoQ.**

The applicant developed a new formulation of sodium benzoate (a granules formulation). The applicant is trying to link (by comparing dissolution profiles) Prohippur to another encapsulated preparation of sodium benzoate however it is unclear how this encapsulated preparation of sodium benzoate can be bridged to the various formulations described in literature. Dissolution data were generated at pH 1.2, 4.5 and 6.8. Data at pH 6.8 is said to be very similar and a convolution approach comparing with an encapsulated product and using literature PK at a low dose, is used to investigate differences in the initial profiles and the expected input on plasma exposure. This approach is not accepted. Dissolution profiles at all pHs should be considered and if a convolution approach is to be used then a IVIVC will need to be demonstrated with a number of formulations showing different initial rates and with clinical PK data. A more detailed description of the PK including the impact of non-linearity at higher doses would also be required **LoQ MO.**

The applicant provides some discussion of the variability of PK data in healthy volunteers, however, subject numbers are limited and not sufficient to allow exploration of any covariates and exposure is compared across different doses with little consideration given to the non-linear PK.

The major concerns are in relation to the variability observed in children. As indicated by Green 1983 the clearance of benzoate varied in one child from 1.56 mL/kg/min to only 0.29 mL/kg/min (- 80%) however no reasons for such variability were identified. In another study (Feoli-Fonseca 1996) the balance of hippurate to benzoate (indicating % of benzoate metabolized to hippurate) was 20-88% of the benzoate dose. A significant intraindividual variation was also reported. For example, one patient (receiving the same dose of benzoate) showed a molar ratio of 0.14 in one study and 0.49 in another. Immaturity of acylation systems in the liver and the kidney in neonates was reported. Taking this information together with the fact that the data supporting the efficacy and safety of sodium benzoate are rather poor (e.g come from a number of heterogenous studies with various methods of administration, posology and combinations of drugs and many of these studies are old) it cannot be concluded that the proposed posology in patients with UCD and NKH is safe.

Some factors which contribute to the variability are discussed: 'Intra individual factors such as dietary glycine intake, availability of acetyl Co-A and/or L-carnitine which are required for the biotransformation of benzoic acid to hippuric acid may be leading to some degree of variability in the response.' But are considered not significant. There appears to be insufficient PK data in patients or healthy volunteers to inform on the expected variability of benzoic acid concentrations and implications for safety and whether a top dose of 750 mg/kg is suitable for all. It is not known if dose adjustments are required in any sub-populations or whether the weight based dose is applicable to children as young as neonates **LoQ MO.**

The proposed posology has been justified based on stoichiometric grounds and without consideration of the PK of sodium benzoate for safety considerations. As indicated by the applicant the proposed maximum dose is based on the dose used in the treatment of adults with hepatic encephalopathy and there is very limited PK data. This justification cannot be accepted for population which include

paediatric patients (including neonates). The fact that the proposed maximum dose is described in the guideline (Häberle 2012) is insufficient. The applicant has discussed the non-linear model in the Kubota paper. It is stated that based on Vmax, the maximum dose should be 500 mg/kg/day, this does not support the higher dose of 750 mg/kg/day. Such a model could be useful to simulate exposure e.g. at higher doses but it should be ensured that Vmax has been adequately captured and differences between HV and patients need to be considered. A population model should be built based on all available data and including the non-linearity in clearance **LoQ MO.**

The applicant has indicated that they have not been able to locate any data on plasma protein binding of sodium benzoate. The partitioning into blood cells however appears to be low. The Assessor is aware of 2 abstracts that suggest they contain data: Effects of pH and tonicity on intestinal permeabilities to blood and to serosal compartment of salicylamide, benzoic acid and other drugs. Oikawa et al, Yakubutsu Dotai (1989),4(6),703-12. Plasma protein binding- lipophilicity relationships: interspecies comparison of some organic acids, Laznicek et al, Journal of Pharmacy and Pharmacology (1987), 39(2), 79-. Plasma protein biding data is considered important to inform doses in special populations e.g. renal impaired or neonates and therefore should be determined if not available **LoQ.**

Clearance values have been calculated for all studies were possible. In some cases there are assumptions used e.g. for other PK parameters which leads to uncertainty on the numbers. It is agreed however that there is a relationship for reducing clearance of benzoic acid as dose increases. This could be better understood with a non-linear model.

There is very limited data at the doses proposed particularly at the high doses'. As mentioned previously, the data in patients is very limited and only single subjects can be compared at similar dose levels. It is suggested that there is similar exposure in adult and children patients however the clearance appears lower in adults

Some differences in PK depending on the gender were noted. The AUC and Cmax were found to be approximately 21% higher in females than in males. The relevant information indicating higher bioavailability of benzoate in females than in males could be added to the SmPC although the limitations of the available data should be also highlighted. No data on weight or race differences in the PK of benzoate are available.

Sufficient information on the mechanism of action was provided. In both indications, NKH and UCD, sodium benzoate acts as a nitrogen scavenger, binding nitrogen in form of glycine (NKH and UCD) or ammonia (UCD), respectively, followed by urinary excretion (Arnstein 1951; Bridges 1970; Barshop 1989; Beyoglu 2012). Sodium benzoate is converted by acylation in the liver into its coenzyme A (CoA) ester, benzoyl-CoA. The latter compound is conjugated to glycine to form hippurate, which is excreted by the kidney. Hippurate contains 1 waste nitrogen atom, so 1 mole of nitrogen is removed for each mole of sodium benzoate administered (Batshaw 1983). In the presence of large amounts of benzoate and glycine, the level of free CoA is a rate limiting factor in the formation of benzoyl-CoA, itself a rate-limiting factor for glycine-N-acylase (Gregus 1992). The increased conjugation of glycine with benzoate tends to concomitantly reduce the glycine pool (Van Hove 1995). Thus, the mechanism of action of sodium benzoate with regards to excretion of nitrogen and thus clearance of ammonia and glycine is via its metabolite - hippurate. In addition to hippurate there are two metabolites of benzoate e.g benzoyl glucoronate and benzoylalanine. The provided data indicate that from 1.5 to up to 12 % of the dose of benzoate can be removed as benzoyl glucoronate and from 0 to 30 % of the dose of benzoate can be removed as benzoylalanine. To reduce the risk of toxicity due to accumulation the plasma levels of sodium benzoate must be measured during therapy and on dose escalation.

A significant variability in hippurate output and benzoate metabolism were observed. The major concerns are in relation to variability observed in children. As indicated by Green 1983 the clearance of

benzoate varied in one child from 1.56 mL/kg/min to only 0.29 mL/kg/min (- 80%) however no reasons for such variability were identified. In another study (Feoli-Fonseca 1996) the balance of hippurate to benzoate (indicating % benzoate metabolized to hippurate) was 20-88% of the benzoate dose. A significant intra-individual variation was also reported. For example, one patient (receiving the same dose of benzoate) showed a molar ratio of 0.14 in one study and 0.49 in another. Immaturity of acylation systems in the liver and the kidney in neonates was reported. Taking this information together with the fact that the data supporting the efficacy and safety of sodium benzoate are rather poor (come from a number of heterogenous studies with various methods of administration, posology and combinations of drugs, many of these studies are old and no GCP compliance was assessed in these studies) it cannot be concluded that the proposed posology in patients with UCD is safe (Please see comments on the MO).The proposed wording to be included in the SmPC is not supported.

The drug can only be given orally without the use of a nasogastric tube. The available data do not support the use a nasogastric tube. The applicant agrees that the formulation cannot be administered by a nasogastric tube or syringe. A protocol is suggested to dissolve the granules, alternatively it is suggested to restrict the indication. It is noted that in line with the described proposed protocol the granules need to be grinded in a coffee grinder. It is very likely that this process will change the PK characteristic of the product. No data on the PK of the grinded granules (as well as on non-grinded granules) were provided by the applicant therefore clinical implications of such changes are unknown

3.3.4. Conclusions on clinical pharmacology

Generally, there remains inadequate data and deficiencies in reporting and discussion of the available PK data regarding absorption, distribution, metabolism and excretion. In addition, adequate data to support the exposure of Prohippur in the proposed population has not been provided. Inadequate information is provided by the applicant to bridge the proposed formulation (a granules formulation) to the various formulations described in literature, therefore, it cannot be concluded that Prohippur has the same PK as these formulations. Therefore, it is difficult to assess whether the proposed posology is either efficacious or safe.

3.3.5. Clinical efficacy

Lucane Pharma has not undertaken any clinical studies to evaluate the biochemical or clinical efficacy of sodium benzoate treatment in patients with NKH or with UCD.

Dose-response studies and main clinical studies

Dosage in NKH patients

(Van Hove 2005) conducted a single dose-finding study in NKH patients. The daily dosage was derived on the basis that one mole of benzoate will be metabolized to one mole of hippurate, together with the estimated nitrogen to be excreted on a restricted protein intake and the glycine intake. The glycine index was calculated as the difference between the molar dose of benzoate needed to normalize plasma glycine levels divided by body weight, reflecting the amount of glycine excreted in urine as hippurate, and the amount of glycine ingested from food. The glycine index reflects the net endogenous synthesis of glycine minus the residual glycine cleavage enzyme activity, hence the endogenous glycine excess. Theoretically if the sum of the glycine synthesis and glycine catabolism by other pathways is constant for weight, then the glycine index reflects the residual activity of the glycine cleavage system, hence the severity of the NKH.

The dose of benzoate required to normalize the steady-state glycine index was evaluated in 10 NKH patients after increasing doses until plasma glycine was in the low-normal range (100–260 µmol/L).

Benzoate was administered orally or by nasogastric tube in 3–6 intakes/d (or by continuous intravenous infusion if enteral administration could not be achieved). Plasma glycine and benzoate levels were obtained 1–2 h after the second dose of the day. The food glycine intake varied from 0.3 to 1.4 mmol/kg/day.

The glycine index was correlated to the severity of the clinical presentation, and it was statistically different (t-test, $p<0.01$) between those who made developmental progress and those who did not. The authors concluded that the glycine index is a stable parameter in an individual patient, but that it differs substantially between patients. This individual variability in the required dose of benzoate has many consequences. When the dose of benzoate exceeds the availability of glycine, and glycine levels are below normal, benzoate has only very limited alternative possibilities for conjugation and excretion, such as the formation of benzoylalanine and benzoylglucuronide (Bridges et al 1970; Shinka et al 1985). Renal excretion of unconjugated benzoate is minimal (Barshop et al 1989). This results in an exponential increase in plasma benzoate levels with increasing benzoate dose resulting in benzoate toxicity. Thus, benzoate treatment in NKH has a narrow, patient-dependent, therapeutic window.

Table 11 Benzoate dose required based on glycine index in NKH patients

Pat.	Onset	Severity	Maximal dev.[al] age or DQ	Anti-convulsants	Glycine index (mmol/kg/d)	Benzoate dose needed (mg/kg/d)			Restrict. Diet needed
						for current Gly index	dietary Gly *(mmol/kg/d)*		
							0.31	*1.42*	
1	1 wk	Severe	<6 wks	3 or more	4.66	672	716	**876**	Gly ; Ser
2	1 wk	Severe	<6 wks		3.88	559	604	**764**	Gly ; Ser
3	2 mo	Severe	<6 wks		3.83	552	597	**757**	Gly ; Ser
4	1 wk	Severe	<6 wks		3.62	522	566	726	
5	6 wk	Severe	<6 wks		4.81	693	738	**898**	Gly ; Ser
6	1 wk	Interm.	6	1 or less	2.54	366	411	571	
7	1 wk	Mod.	23		0.92	133	177	337	
8	1 wk	Mod.	21		1.90	274	318	478	
9	1 wk	Mild	30		1.80	259	304	464	
10	1 y	Mild	55		1.51	218	262	422	

Developmental Quotient (DQ) is a ratio of the functional age to the chronological age. It is a means to simply express a developmental delay. DQ = ((developmental age) / (chronological age)) * 100. The dietary glycine intake varied between 0.31 and 1.42 mmol/kg/d.

Table 26 Effect of daily benzoate dose to plasma glycine levels in NKH patients

Reference	Patient	Benzoate dose (mg/kg/d)*	Plasma glycine level (µmol/L)	
			before	after
Baumgartner 1969	1	500	893	533
Krieger 1977	1	1200 mg/d	629	329
	2	900 mg/d	855	520
Matalon 1983	1	125	1076	704
	2		721	654
Hamosh 1992	1	500	943	689
		750	689	<323
Van Hove 1995	1	500-750	795	83-320
	2	500	897	917
		640	917	82-352
		750	113-235	<341
Hamosh 1998	4	500-750	807	83
Van Hove 1998	3	683	815	399
	4	743	1659	632

Lu 1999	1	250	725	535
	2	300	474	170
Van Hove 2000	3	480	772	500-550
Wiltshire 2000	1	250	652-642	369
Chien 2004	1	250-300	2097	1338
	2	300	1185	425
	4	500	812	415
	5		669	559
	6		2110	121
Korman 2004	1	250	1200	364-475
	2	250	907-2285	492
Suzuki 2010	1	150	1274	310-912

(*) except where indicated

Dosage in UCD (or LPI) patients

No specific study has been conducted. However, the applicant suggests that a dose between 1.5-1.8 mmol/kg, given iv in 90 min during a hyperammonaemic crisis is safe based on previous published data (Simell 1986). The applicant considers that in UCD (or LPI) patients the benefit of sodium benzoate resides in its ability to add a vehicle of waste nitrogen synthesis and to suppress urea synthesis as on a molar basis hippurate is less effective at eliminating excess waste nitrogen by a factor of two compared to urea (which contains two nitrogen moles/mole). For ammonia levels exceeding 250 µmol/L, the response being stoichiometric, the dose of benzoate would be limited by its potential toxicity (Batshaw 2001). It would appear that a dose of 2.0 mmol/kg (288 mg/kg) benzoate is likely to cause symptoms of overdose (dizziness, nausea, vomiting) in the majority of subjects (Simell 1986).

In the neonatal period, induction of hippurate synthesis may be delayed and plasma benzoate concentrations may reach potentially toxic concentrations. The variability of benzoate metabolism in infants and children may result from immaturity of acylation systems in the liver and the kidney. Between birth and 7 months of age, hepatic mitochondrial N-acetylase activity represents 5-41% of the mature activity which is reached by 18 months of age (Mawal 1997). This highlights the importance of monitoring benzoate plasma levels to avoid toxicity (Feillet 1998). In chronic oral treatment of UCD patients the dose of benzoate is usually 250 mg/kg/d (Brusilow 1996).

Main studies

No studies have been conducted by the applicant. Literature references are provided to support efficacy.

Efficacy of benzoate in the treatment of UCD

The applicant has not identified the studies they regard as pivotal. The main study appears to be the one conducted at The John Hopkins hospital in the utilization of alternative waste nitrogen pathways in addition to the diet as a prospective therapeutic protocol under a US-IND. This protocol has been amended several times over its 15-year duration.

Table 1 Principal clinical trials in UCD supporting the US-IND

Ref.	Deficient enzyme	Period I Dates Number of subjects/deaths Duration (range)	Period IIa Dates Number of new subjects/deaths Duration (range)	Period IIb Dates Number of new subjects/deaths Duration (range)	Period IIIa Dates Number of new subjects/deaths Duration (range)	Period IIIb Dates Number of new subjects/deaths Duration (range)
Maestri 1991	CPS-I OTC ASS	1981-1984 1/0 2/2 1/0 (0.3-4.0y)	1984-1987 3/0 3/0 3/0 (0.25-6.3y)		1987-1988 3/1 1/0 3/0 (0.83-4.0y)	N/A
Maestri 1995	ASS	1979-1983 14/0 2.2 y (0.06-5.6)	1984-~1987 9/3 3.4 (0.17-7.3)	~1987-1989 9/0 1.2 (range ns)	1989-1990 7/1 3.7 (range ns)	1990-1995 19/3 3.9 (range ns)
Maestri 1996	OTC	1980-1984 11 Duration ns	1984-1987 22 Duration ns		1987-1996 28 Duration ns	

ns: not specified; duration represents time under IND protocol period or phase for each patient
The same patient may be overlapping several consecutive periods of the IND protocol
In Maestri 1991 and Maestri 1996, subparts a and b of periods II and III are merged, respectively
The date limits of each period may be fluctuating from one patient to the other. These are the most probable dates from the literature sources.
Period I sodium benzoate 250 mg/kg/d
Period IIa sodium benzoate 250 mg/kg/d + sodium phenylacetate 250 mg/kg/d
Period IIb sodium benzoate 250 mg/kg/d + sodium phenylacetate or sodium phenylbutyrate 250-300 mg/kg/d
Period IIIa sodium phenylacetate 250-300 mg/kg/d
Period IIIb sodium phenylbutyrate 450-600 mg/kg/d

In the three main clinical references (Maestri 1991, Maestri 1995 and Maestri 1996), patients with UCDs [CPS-I, OTC or ASS deficiencies] were included. Until 1987 - 1989, depending on the reference, sodium benzoate was used alone as the waste nitrogen scavenger; it was then used in combination with phenylacetate. Afterwards, benzoate was discontinued in favour of phenylacetate and/or phenylbutyrate at higher doses.

The first treatment program (1985-1994) consisted of 162 patients, of whom 148 patients were evaluable (87 with prior therapy and 61 without prior therapy). Of those, 99 patients suffered from OTC, 31 from ASS patients and 18 from CPS. 55% of patients were less than twelve years old at the time of the last visit to the investigator.

Table 15 Principal clinical trials in UCD supporting the US-IND

Ref.	Deficient enzyme	Period I Dates Number of subjects/deaths Duration (range)	Period IIa Dates Number of new subjects/deaths Duration (range)	Period IIb Dates Number of new subjects/deaths Duration (range)	Period IIIa Dates Number of new subjects/deaths Duration (range)	Period IIIb Dates Number of new subjects/deaths Duration (range)
Maestri 1991	CPS-I OTC ASS	1981-1984 1/0 2/2 1/0 (0.3-4.0y)	1984-1987 3/0 3/0 3/0 (0.25-6.3y)		1987-1988 3/1 1/0 3/0 (0.83-4.0y)	N/A
Maestri 1995	ASS	1979-1983 14/0 2.2 y (0.06-5.6)	1984-~1987 9/3 3.4 (0.17-7.3)	~1987-1989 9/0 1.2 (range ns)	1989-1990 7/1 3.7 (range ns)	1990-1995 19/3 3.9 (range ns)
Maestri 1996	OTC	1980-1984 11 Duration ns	1984-1987 22 Duration ns		1987-1996 28 Duration ns	

ns: not specified; duration represents time under IND protocol period or phase for each patient
The same patient may be overlapping several consecutive periods of the IND protocol
In Maestri 1991 and Maestri 1996, subparts a and b of periods II and III are merged, respectively
The date limits of each period may be fluctuating from one patient to the other. These are the most probable dates from the literature sources.
Period I sodium benzoate 250 mg/kg/d
Period IIa sodium benzoate 250 mg/kg/d + sodium phenylacetate 250 mg/kg/d
Period IIb sodium benzoate 250 mg/kg/d + sodium phenylacetate or sodium phenylbutyrate 250-300 mg/kg/d
Period IIIa sodium phenylacetate 250-300 mg/kg/d
Period IIIb sodium phenylbutyrate 450-600 mg/kg/d

In all studies under the US-IND protocol (Table 15) treatment of neonatal hyperammonaemic episodes or pre-symptomatic treatment of pre-identified subjects included priming and 24h-continuous infusion with sodium benzoate and sodium phenylacetate, 250 mg/kg each, until medications could be given orally, however any carry-over effect of these neonatal procedures was not considered.

Thirty-two patients (6 CPS, 20 OTC, 4 ASS, 2 ASL) identified prenatally as being at risk for a UCD were treated presymptomatically from birth (Maestri 1991). Twelve (2 ASL, 4 ASS, 1 CPS, 5 OTC) out of the 15 symptomatic infants survived the neonatal period, so the protocol was successful in averting neonatal hyperammonaemic coma in all except in 3 patients with OTC (the 17 other infants were unaffected and their medication discontinued within 96 h). There were 3 further deaths (1 CPS, 2 OTC) among these 12 children, all during a hyperammonaemic episode. In one the high frequency of hyperammonaemic episodes (9 episodes in his 7 months' life) suggested that nitrogen metabolism was not adequately controlled by benzoate alone.

The global survival rate at 2 years was 73%, and even 100% in ASS (which compared favourably to the 90% survival rate in this population from unpublished results, Maestri 1991). Under benzoate treatment alone (250 mg/kg/d) one pre-symptomatically treated patient (OTC) died after 7 months of treatment. Survival rate on single treatment with benzoate was 3 out of 4 patients (75%). With benzoate and phenylacetate one pre-symptomatically treated patient (OTC) died after 33 months' treatment. Survival rate on combined benzoate and phenylacetate treatment was 6 out of 10 patients (60%). On treatment with sodium phenylacetate or sodium phenylbutyrate one pre-symptomatically treated patient (CPS) died after 46 months' treatment. Survival rate on treatment with sodium phenylacetate or sodium phenylbutyrate was 6 out of 7 patients (85%). The authors considered that survival was improved with phenylbutyrate.

Long-term survival was also evaluated in 24 children with ASS receiving waste nitrogen excretion activation protocols: 23 infants born between January 1979 and September 1989 rescued from hyperammonaemic coma from ASS with neonatal onset and 1 boy born in 1976 included at 5 years of age (Maestri 1995). The cumulative survival rate at five years was 87.5%, and 73.7% at ten years.

A total of fourteen patients have been maintained on single treatment with oral sodium benzoate (250 mg/kg/d) for an average of 2.2 years (0.06-5.6 years) during which no death occurred. Therefore, survival was 100% at 2 years on single benzoate treatment. These data are consistent with earlier results in patients with ASS (Maestri 1991). Twenty-three patients have been treated with oral sodium benzoate and sodium phenylacetate (250 mg/kg/d each) for an average of 3.4 years (0.17-7.3 years) during which 3 deaths occurred (13%). Nineteen patients have been treated with sodium phenylacetate or sodium phenylbutyrate (450-600 mg/kg/d) for an average of 3.9 years during which 4 deaths occurred (21%).

Among 17 long-term survivors, 1 had received an orthotopic liver transplant, 2 were withdrawn from the study and treated with a product including benzoate and phenylacetate and 14/19 patients were still under treatment with high doses (450-600 mg/kg/d) sodium phenylacetate or sodium phenylbutyrate.

A total of 7 patients died during the follow-up of the study. Out of these 17 patients, none had followed the single benzoate treatment protocol, 3 had followed the benzoate and phenylacetate protocol, 1 had followed the high dose sodium phenylacetate protocol, 2 had followed the high dose phenylacetate or phenylbutyrate protocol and one the high dose phenylbutyrate protocol.

Long-term survival was also studied in 39 OTC girls enrolled between 1979 and 1990 to receive a waste nitrogen excretion activation protocol. Seven withdrew before the 5 year-follow-up and among the 32 remaining 3 died (9%): 2 of whom during a hyperammonaemic episode one of which after 5 year-treatment. Therefore, global survival at 5 years was 94% (Maestri 1996). Historical data

(Batshaw 1986) report a death rate of 9/61 (14%) in untreated heterozygote female OTC i.e. a survival rate of 85% which compares with the 94% five-year survival under waste nitrogen excretion activation protocols.

Table 16 US-IND studies summary survival

Ref.	Time point of survival	Diagnosis	Patients	Survival %			
				Benzoate alone	Benzoate + PA	PB	other procedure
Batshaw 1980	Immediate	ASS OTC CPS	7	71	-	-	EXT/PD/HD 52/56 on 37/44 (references) 14
Maestri 1991	2 years	ASS OTC+CPS	3 5+3	100 75	100 60	100 85	90 -
Maestri 1995	2 years	ASS	24	100	87	79	-
Maestri 1996	5 years	OTC	32	94			85 on 61 untreated (Batshaw 1986)

EXT exchange transfusion, HD hemodialysis, PA phenylacetate, PB phenylbutyrate, PD peritoneal dialysis

Apart from the US-IND, several clinical reports and studies reported the beneficial effect of sodium benzoate in the long-term treatment of UCD patients. The table below provide a summary of outcomes in UCD treated long term:

Table 14 Outcome in UCD patients treated on a long-term basis with benzoate

Ref.	Deficient enzyme	N of patients	Age Gender	Sodium benzoate dose	Outcome
Brusilow 1979	CPS	1	16 y F	6.5 g/d 10 days	55 % increase in urinary nitrogen excretion Significant fall in plasma ammonia and plasma glutamine
	ASS	1	3 F	1.2 g/d	ns
Brusilow 1980	CPS	1	17 y F	6.5 g/d 11 days (160 mg/kg/d)	35 % increase in total urinary nitrogen excretion (26% in the form of hippurate) (4.8±0.7→6.5±0.4 mg/mg creatinine) Plasma ammonia 29.5±1 µmol/L →22.9±2 µmol/L ($p<0.02$)
Batshaw 1982	CPS	2	18 M 14 M	250 mg/kg/d	Both alive IQ 80-100 Hippurate nitrogen urinary excretion 37-48%
	OTC	7	18 M 22 M 11 M 8 M 10 M 8 M 8 M		3/7 died IQ >68:4/7; 52-68:0/7; <52:3/7 Hippurate nitrogen urinary excretion 28-31%
	ASS	7	42 F 34 M 27 F 19 F 19 F 10 F 8 F		1/7 died IQ >68:1/7; 52-68:4/7; <52:2/7 Hippurate nitrogen urinary excretion 11-23%
	J. Hopkins' subgroup	9			Mean (SE) Plasma ammonia decreased from 997±249 µmol/L to 35.5±1.9 µmol/L Plasma glycine normal 190.6 ±11.9 µmol/L Plasma benzoate 1.5 mmol/L; Hippuratemia 0.7 mmol/L
Call 1984	CPS	1	33 y F	410 mg/kg/d x 9 mo	Plasma ammonia range 100-420 µmol/L→70-100 µmol/L Disappearance of symptoms (ataxia, giddiness, somnolence, nausea, anorexia). IQ 73-74

Guibaud 1984	OTC	1	3 d M	300 mg/kg/d x 30 mo	Disappearance of symptoms (lethargy, plasma ammonia range 714-819 µmol/L) →clinically normal on D10
Qureshi 1984	ARG1D	1	15.5 y F	Control (diet)	Progressive spastic diplegia, IQ85, anorexia Plasma ammonia 76±5 µmol/L Urea nitrogen urinary excretion 35.1±2.1% Hippurate nitrogen urinary excretion 1.5±0.1%
				7.6 g/d x 1 wk	Plasma ammonia 54±5 µmol/L Plasma arginine 550±21 µmol/L Urea nitrogen urinary excretion 21.7±1.4% Hippurate nitrogen urinary excretion 19.6±1.8%
				Inadvertent stop x 24 h	Lethargy, vomiting, coma Plasma ammonia 263 µmol/L
				11.4 g/d x 9 mo	Plasma ammonia 31±5 µmol/L Plasma arginine 319±13 µmol/L Urea nitrogen urinary excretion 12.7±0.7% Hippurate nitrogen urinary excretion 35.4±1.9%
				Idem+arginine restriction	Improved nutritional status, BW gain 3.5 kg/9 months stopped progression of spastic diplegia Plasma ammonia 31±5 µmol/L Plasma arginine 161±14 µmol/L Urea nitrogen urinary excretion 4.3±0.4% Hippurate nitrogen urinary excretion 42.6±4.2%
Van de Bor 1984	CPS	1	4 d F 7 months	250 mg/kg/d x 3 wks	Plasma ammonia 158-210 µmol/L →Normal 40-100 µmol/L Plasma glutamine 1800 µmol/L→ Normal 400-800 µmol/L Within 3 wks→ benzoate withdrawn: new increase in ammonia 180 µmol/L Reinstitution of benzoate→ normal
Letarte 1985	OTC	1	7 y M	250 mg/kg/d x 3 y	IQ 62-70, no HA crisis, improved growth & P. intake Plasma ammonia decreased: 74 → normal (≤50 µmol/L) Hippuraturia 17-32 mmol/d (15-20% excreted nitrogen) Plasma benzoate 0 mmol/L; Hippuratemia 0.015 mmol/L
Walter 1992	ASS	1	4 d M	500 mg/kg/d IV	Plasma ammonia 2500 µmol/L→1560, 420, <100 µmol/L on day 5, day 6 and day 9 and x 1y under NaPB+BNA at 13 mo convulsion crisis with plasma ammonia 381 µmol/L →113 µmol/L
Connelly 1993	OTC	2	9 F	Not specified x 4 mo	Good developmental, mood progress, and weight gain Plasma ammonia: 220 µmol/L → 66 µmol/L (normal <50µmol/L) in 6 d
			5 F	Not specified x 11 y	Improved and stable Plasma ammonia: 236 µmol/L→? at 11 y new encephalopathy episode (plasma ammonia 230 µmol/L)
Al-Hassnan 2008	HHH	1	4 y F	250 mg/kg/d x 38 mo	Plasma ammonia: 532 µmol/L→42 µmol/L Plasma ornithine: 465 µmol/L→120 µmol/L
					Urine homocitrulline: 108 →23 mmol/mol creat
De Groot 2010	ASS with acute liver failure presentation	2	17 mo F	Not specified	Transaminases and LFT normalized with 3 wks Plasma ammonia: 114 µmol/L→normal within a few days
			22 mo M	Not specified	LFT and plasma ammonia (210 µmol/L) normalized within 2 days
Ko 2012	LPI	1	3.7 y F	200 mg/kg/d x 1 y	Plasma ammonia: 520 µmol/L→Normal No decompensation after treatment initiation
Bergmann 2014	OTC	1	8 y M	5.5 g/m^2	Plasma ammonia: 1351-1561 µmol→HD→368 µmol/L→ SB+SP IV→93 µmol/L→PO from D8 →<9 µmol/L1 from D10

Other studies conducted in UCD patients:

Martin-Hernandez 2014

- Spanish Observational, cross-sectional and multicentre study. Clinical, biochemical and genetic data were collected from patients with UCDs, treated in the metabolic diseases centres in Spain between February 2012 and February 2013,
- 1-year biochemical data was collected at clinical onset in symptomatic patients (N=91) and at last follow-up in all patients (N=96)
- Results: 104 patients from 98 families were included. Ornithine transcarbamylase deficiency was the most frequent condition (64.4%) (61.2% female) followed by type 1 citrullinemia (21.1%) and argininosuccinic aciduria (9.6%). Only 13 patients (12.5%) were diagnosed in a pre-symptomatic state. 63% of the cases presented with type intoxication encephalopathy. The median ammonia level at onset was 298 µmol/L (169-615). The genotype of 75 patients is known, with 18 new mutations having been described. During the data collection period four patients died, three of them in the early days of life. The median current age is 9.96 years (5.29-18), with 25 patients over 18 years of age. Anthropometric data, expressed as median and z-score for the Spanish population is shown. 52.5% of the cases present neurological sequelae, which have been linked to the type of disease, neonatal onset, hepatic failure at diagnosis and ammonia values at diagnosis. 93 patients are following a protein restrictive diet, 0.84 g/kg/day (0.67-1.10), 50 are receiving essential amino acid supplements, 0.25 g/kg/day (0.20-0.45), 58 arginine, 156 mg/kg/day (109-305) and 45 citrulline, 150 mg/kg/day (105-199). 65 patients are being treated with drugs: 4 with sodium benzoate, 50 with sodium phenylbutyrate, 10 with both drugs and 1 with carglumic acid.
- 68% of the symptomatic patients had received nitrogen scavenging drugs, only a few had sodium benzoate either alone (4) or a combination product (10).

Rüegger 2014

- European population-based study. Cross sectional observational study of 208 patients with non-classical urea cycle disorders. Cohort of 208 patients, largest subgroup was 121 patients with x-linked OTC deficiency, of whom 83 were female and 29% of these asymptomatic.
- Results:

Table 4 Nitrogen scavenger drugs and L-citrulline and L-arginine

Patients (clinical course)	Nitrogen scavenger drugs (n=196)					L-citrulline and L-arginine supplementation (n=199)				
	SB	SP	SD & SP	None	Total	Arg	Cit	Cit & Arg	None	Total
Asymptomatic	3 (5%)	1 (1%)	3 (5%)	57 (89%)	64 (100%)	18 (27%)	5 (8%)	3 (4%)	41 (61%)	67 (100%)
Symptomatic	41 (31%)	25 (19%)	29 (22%)	36 (28%)	131 (100%)	68 (52%)	37 (28%)	7 (5%)	19 (15%)	131 (100%)
Unknown	1	–	–	–	1	1	–	–	–	1
Total	45 (23%)	26 (13%)	32 (16%)	93 (48%)	196 (100%)	87 (44%)	42 (21%)	10 (5%)	60 (30%)	199 (100%)

Data on treatment with nitrogen scavenger drugs and L-citrulline and L-arginine supplementation for patients with asymptomatic, symptomatic or unknown clinical courses

SB sodium benzoate; *SP* sodium phenylbutyrate; *Cit* L-citrulline; *Arg* L-arginine

The Ruegger 2014 paper did not discuss efficacy of any of the treatment protocols.

In rarer indications, such as ASL (Grioni 2011, Kalkan Uçar 2015), HHH (Al-Hassnan 2008, Martinelli 2015) or LPI (Ko 2012, Mauhin 2017) case reports have been presented showing the effectiveness of treatment including protein restricted diet and oral sodium benzoate, although this experience is very heterogeneous.

Efficacy of benzoate in the treatment of NKH

The applicant has conducted a review of the literature using Medline together with the available literature data from Professor Joerg Breitkreutz (Düsseldorf, Germany) in 2014. All publications providing individual data on NKH patients' clinical and/or biochemical condition before and/or after benzoate administration(s) were selected. Reviews were added when they provided individual patients details (e.g. Van Hove 2005, Hennermann 2012). Duplicates were eliminated; though, any relevant complementary information on patients' data found in a second publication was added to the results.

Demographic characteristics: a total of 88 cases who had received benzoate treatment were reported in the literature (Ziter 1968, Baumgartner 1969, Trijbels 1974, Krieger 1977, Von Wendt 1981, Matalon 1983, Cole 1985, Luder 1989, Hamosh 1992, Zammarchi 1994, Van Hove 1995, Alemzadeh 1996, Boneh 1996, Arnold 1997, Hamosh 1998, Van Hove 1998, Al-Essa 1999, Lu 1999, Maeda 2000, Neuberger 2000, Randak 2000, Van Hove 2000, Wiltshire 2000, Korman 2002, Aliefendioglu 2003, Korman 2004, Kure 2004, Chien 2004, Tsao 2005, Zenciroglu 2005, Korman 2006, Chiong 2007, Demirel 2008, Mastrangelo 2008, Rossi 2009, Yis 2009, Suzuki 2010, Tsao 2010, Dhamija 2011, Chiu 2015, Bjoraker 2016), representing a total of 86 individual subjects, as 2 patients have been reported twice. A total of 70/86 patients (81%) published in the single case reports showed improvement of one or several clinical symptoms, i.e. there appeared to be a positive clinical effect of benzoate treatment which has also been evidenced in general reviews (Hennerman 2012).

The applicant also appears to have added data for some points from a review by Swanson 2015. The applicant states that out of the individual published 86 cases who were receiving treatment with sodium benzoate, a total of 150 values of plasma glycine and CSF glycine levels were identified. For 120 of these values the dose of benzoate was specified. In addition, a recent review of 124 patients with NKH provides in online Supporting Information their individual glycine values (Swanson 2015), thus generating a total database of ~260 paired plasma and CSF glycine values. The applicant has not provided data to conclude that plasma and CSF glycine levels are correlated. Comparing the highest value before and the lowest one obtained after/under treatment with sodium benzoate, and for this latter treatment the highest dose documented, a total of 41 pairs of plasma glycine values in a median (range) 4 (0.03-60) months treatment before/after the treatment were obtained. They show that sodium benzoate was effective in reducing glycine levels in plasma by a mean of 630 ± 72 µmol/L, reaching normal values in 27 cases.

In the review by Hennermann 2012 a positive biochemical effect was defined as a reduction in plasma glycine to ≤300 μmol/L which was obtained in 31/45 patients.

With regards to clinical benefits the applicant presents data from their own review of 86 single case reports and the survey conducted by Hennermann 2012. They also at one stage refer to another survey by Hoover-Fong 2004 in neonates.

A total of 70/86 patients (81%) published in the single case reports showed improvement of one or several clinical symptoms, i.e. there appeared to be a positive clinical effect of benzoate treatment. Forty patients had reduced seizures/myoclonic jerks and 27 patients with lethargy/poor interaction showed an improved alertness, leading to a cumulative "positive effect" of benzoate on these 2 parameters in 44/86 patients (51%). In retrospective surveys, both, lower and higher numbers of patients who showed an effect of benzoate treatment to clinical symptoms were reported: increased alertness and/or decreased seizures in 16/39 neonates (41%) in the survey by Hoover-Fong et al.

(2004), and 81% (31/38) in the survey by Hennermann et al. (2012). This may be accounted for by the heterogeneity of clinical data, follow-up period and non-systematic reporting and by the fact that the number of anticonvulsants (which is not reported in sufficient detail in most published case reports) was not always considered in the data analysis.

Table 23 Developmental/cognitive evaluations under benzoate in NKH patients

Reference	Patient id.	Benzoate dose (mg/kg/d)*	Outcome timepoint	Development outcome
Von Wendt 1981	1	150-400	ns	PM retardation
Matalon 1983	1	125	7 months	Severe DD
	2		8 months	Severe DD
Cole 1985	1	5 g/d	12 years	DD speech impediment
	2		10 years	Subnormal IQ significant difference between receptive and expressive verbal activity
	3		8 years	Marked speech DD, motor delay
Hamosh 1992	1	500-750	12.5 months	DD (7-8 months of age) DQ 55-65
Van Hove 1995	2	500-640	3.8 years	Motor skills 4-16 weeks of age
	4	500	5-8 months	DD
	5		9 months	DD (3-4 months of age)
Alemzadeh 1996	1	400	13 months	DD (6 months of age)
Boneh 1996	1	250	5 years	DD (5 months of age)
	3		3 years	Slow PM development
	4		2.2 years	*Normal PM development*
	5		14 months	DD (7 months of age)
	6		10 months	DD (5-6 months of age)
Hamosh 1998	1	500-750	60 months	DD (18-24 months of age) DQ 30-40
	2		48 months	DD (8 months of age) DQ 19
	3	500	27 months	DD (9 months of age) DQ 33
Lu 1999	1	250	3 months	Severe PM retardation
	2	300	3 months	Severe PM retardation
Neuberger 2000	1	250-750	20 months	DD (12 months of age)
			24 months	DQ 62-79
			33 months	DQ 48-61
Randak 2000	3	500	ns	Severe PM and mental retardation
Van Hove 2000	2	750	5.5 years	DD (3-6 months of age)
	3	480	4.5 years	DD poor dev. outcome
Wiltshire 2000	1	250	6 months	DBC total score 94%tile→42%tile
Korman 2002	1	ns	24 months	Significant DD
Aliefendioglu 2003	1	250	8 months	Grossly retarded
Zenciroglu 2005	1	ns	8 months	DD (1 month of age)
Van Hove 2005	1-5	ns	ns	PM development <6 weeks
	6		>2 years	PM development 8 months, DQ 6
	7-10			DQ 23- 21- 30- 55
Korman 2006	2	ns	11 months	Severe PM retardation
Chiong 2007	1	250	7 years	Borderline normal IQ, speech normal Major motor retardation
Demirel 2008	3	750	15 months	Mental & motor retardation
Mastrangelo 2008	1	250-500	11 years	Severe PM & cognitive impairment
Suzuki 2010	1	150	10 months	DD (5-6 months of age)
Tsao 2010	1	600	6 years	Profound mental retardation
	2		6.5 years	Profound mental retardation
Dhamija 2011	1	ns	4 months	No social smile
Bjoraker 2016	7	ns	1A 11 y 2 mo	DQ 37

			1B	9 y	DQ 51
			2A	4 y 9 mo	DQ <20
			2B	2 y 5 mo	DQ 48
			3A	5 y	IQ 44
			3B	1 y 2 mo	IQ 55
			4B	8 y 3 mo	IQ 75

(*) except where indicated, %tile percentile, DBC development behavior checklist, DD developmental delay, DQ development quotient, IQ intellectual quotient, PM psychomotor, ns not specified. In Bjoraker 2016, patient 4A died at 13 months.

There is limited data to suggest that treatment with sodium benzoate and dextromethorphan improves neurocognitive outcome when instituted early. In 4 sibling sets from 4 different families, where the 1st affected child was diagnosed and treated after 2-6 months whereas the 2nd affected child was diagnosed prenatally and treated effectively from birth or the 1st week, with a dose of benzoate that was sufficient to control plasma glycine levels (<400 µmol/L) and dextromethorphan, that these children progressed substantially better than their siblings who had been late treated (Bjoraker 2016). Benzoate therapy does not appear to be associated with improved psychomotor development.

Supportive studies

Type of study	Reference	Subjects		Exposure	
		Type	N	Duration	Daily dose (mg/kg) (except where indicated)
PK-PD	Green 1983	UCD	3	-	500
	Simell 1986	LPI	5	-	288
	Oyanagi 1987	UCD	2	-	130, 150
	Barshop 1989	UCD	2	-	180-650
		NKH	9		
	Feoli-Fonseca 1996	UCD	11	-	ns
Clinical	Brusilow 1979	UCD	2	10 d	1.2 g, 6.25 g
	Brusilow 1980	UCD	1	11 d	160
	Batshaw 1980	UCD	7	< 2 d	250-500
	Batshaw 1982	UCD	26	7-62 mo	250-500
	Batshaw 1983	UCD	9	-	250
			1		800
	Brusilow 1984	UCD	7	< 4 d	250-500
	Msall 1984	UCD	26		250
	Call 1984	UCD	1	9 mo	410
	Guibaud 1984	UCD	1	30 mo	250-500
	Qureshi 1984	UCD	1	2 y	250-375
	Van de Bor 1984	UCD	1	15 mo	250
	Letartre 1985	UCD	1	3 y	250
	Batshaw 1988	UCD	1	8 y	200-375
	Maestri 1991	UCD	32	4.5 y mean	250
	Walter 1992	UCD	1	1.5 y	150-500
	Connelly 1993	UCD	2	5.8 y mean	ns
	Maestri 1995	UCD	24	5.6 y mean	250
	Renner 1995	UCD	1	5 y	200
	Maestri 1996	UCD	39	7.5 y max	250
	Uchino 1998	UCD	216	-	ns
	Bachmann 2003	UCD	44	-	ns
	Enns 2007	UCD	299	ns	250
	Al-Hassnan 2008	HHH	1	3 y	250
	De Groot 2010	UCD	2	3 wks max.	ns
	Grioni 2011	UCD	4	6.25 y mean	55-125
	Ko 2012	LPI	1	1 y	200
	Bergmann 2014	UCD	1	1 y	ns
	Boneh 2014	UCD	28	ns	ns
	Kalkan Uçar 2015	UCD	1	5.75 y	500
	Martin-Hernandez 2014	UCD	14	ns	165±80.5 to 184±92.3
	Rüegger 2014	UCD	77	ns	ns
	Martinelli 2015	HHH	26	ns	ns
	Blair 2015	UCD	1	1 year	ns
	Husson 2016	UCD	61	2 d median	150-606
	Unsinn 2016	UCD	45	ns	ns
	Ziter 1968	NKH	1	3 y	500
	Baumgartner 1969		1	7 mo	ns
	Trijbels 1974		1	3 y	100-300
	Krieger 1977		2	5 mo mean	225-500
	Von Wendt 1981		1	-	150-400
	Carson 1982		31 max	ns	ns
	Matalon 1983		2	1 y mean	125

Type of study	Reference	Subjects		Exposure	
		Type	N	Duration	Daily dose (mg/kg) (except where indicated)
	Cole 1985		3	ns	5 g/d
	Luder 1989		1	1 y	ns
	Hamosh 1992		1	1 y	500-750
	Zammarchi 1994		1	5 mo	500
	Van Hove 1995		5	3 y mean	500-750
	Alemzadeh 1996		1	10.5 mo	400
	Boneh 1996		6	2.8 y mean	250
	Arnold 1997		2	5.6 y mean	250-750
	Hamosh 1998		3	2.33 y mean	500-750
	Van Hove 1998		3	5 y mean	250-750
	Al-Essa 1999		1	3 wk	500
	Lu 1999		2	10 mo mean	250-300
	Maeda 2000		1	3 y	ns
	Neuberger 2000		1	26.5 mo	250-750
	Randak 2000		3	ns	500
	Van Hove 2000		3	2.1 y mean	480-500
	Wiltshire 2000		1	ns	250
	Korman 2002		1	17 mo	ns
	Aliefendioglu 2003		2	5.5 mo mean	250
	Chien 2004		5	3 y mean	250-500
	Hoover-Fong 2004		57	ns	ns
	Korman 2004		3	1.3 y mean	250
	Kure 2004		2	3.5 y mean	ns
	Roy 2004		3	ns	500
	Van Hove 2005		10	-	750
	Zenciroglu 2005		2	13 mo mean	ns
	Korman 2006		1	11 mo	250
	Chiong 2007		1	7 y	ns
	Demirel 2008		5	6.8 mo mean	250-750
	Mastrangelo 2008		1	11 y	250-500
	Rossi 2009		1	48 h	ns
	Yis 2009		1	1.5 y	750
	Suzuki 2010		1	9 mo	150
	Tsao 2005, 2010		2	6.25 y mean	600
	Dhamija 2011		1	4 mo	ns
	Hennermann 2012		38	ns	200-750
	Chiu 2015		1	1 mo	250-750
	Swanson 2015		113	ns	ns
	Bjoraker 2016		8	5.2 y mean	ns

CT: clinical trial; d: day; h: hour; LPI: lysinuric protein intolerance; mo: months; NKH: non ketotic hyperglycinemia; ns: not specified; UCD: urea cycle disorders; wk: week; y: years

3.3.6. Discussion on clinical efficacy

This application for an indication in UCD and NKH was supported by bibliographic data. There remains still uncertainly about the quality and completeness of evidence provided. The reviewed articles were cross-referenced against existing documentation, and there is no evidence to indicate that the existing documentation is complete. Important information such as information sources with appropriate dates of coverage, study selection (screening and assessment of eligibility), and quality of assessment are missing. A robust discussion to demonstrate how identification of relevant studies from a number of different sources (including unpublished sources) has not been conducted, how the selection of studies for inclusion was made and importantly the discussion would include an evaluation of the strengths and limitations on these studies, preferably based on clear, predefined criteria was expected but it was not provided. The quality and scope of published literature varies widely. The strength of the conclusions

and the ability to provide decision-makers with reliable information depends on the inclusion of reviews that meet a minimum standard of quality, in line with GCP requirement. As there was no critical appraisal of the data in line with the GCP requirements the quality of studies used to support this application cannot be assured. Reporting and publication bias are potential concerns given the small number of cases. Thus it is not clear whether the evidence presented is complete and the studies used to support of this application are of sufficient quality **LoQ MO.**

The proposed therapeutic indications are: Prohippur is indicated as adjunctive therapy in the chronic management of non ketotic hyperglycinemia, as well as that of urea cycle disorders including carbamoyl-phosphate synthase-1 deficiency, ornithine transcarbamylase deficiency, citrullinaemia type 1, argininosuccinic aciduria, hyperargininaemia, n-acetylglutamate synthase deficiency, ornithine translocase deficiency and lysinuric protein intolerance.

The applicant cannot provide the data supporting the use of benzoate as the sole treatment. In the majority of studies provided by the applicant benzoate was used in combination of other nitrogen-scavenging therapies or dialysis. This makes the assessment of the role of sodium benzoate difficult.

The applicant has provided tables of patients who apparently have received chronic therapy with sodium benzoate. It is not clear whether the column titled duration was duration of oral therapy and this requires clarification from the applicant. The tables provided are for the treatment of CPS, OTC, ASS (Citrullinaemia), ASL (argininosuccinic aciduria), ARG1 (hyperargininaemia) and HHH. There have been no tables provided for n-acetylglutamate synthase deficiency, ornithine translocase deficiency and lysinuric protein intolerance and non ketotic hyperglycinemia which are listed in the proposed indication.

The applicant provided the data for each type of UCD disorders e.g.:

- CPS deficiency: 12 patients, age from 2 days to 33 year, dose of sodium benzoate from 114 to 500 mg/kg/d, outcome of 5 patients: deaths or not specified
- OTC deficiency: 103 patients, age from 8 days to 17 years, dose of sodium benzoate from 250 mg to 300 mg mg/kg/d, outcome 13 deaths. It need to be noted the biggest publication provided to support this indication (Ruegger 2014) is the retrospective collection from 20 metabolic centres and the dose of sodium benzoate is not specified.
- ASS deficiency: 41 patients, age from 4 days to 42 months, the dose of sodium benzoate from 150 to 250 mg mg/kg/d
- ASL deficiency: 21 patients, age 1 day to 62 months, the dose of sodium benzoate from 55 mg to 500 mg mg/kg/d
- ARG1D: 11 patients, age from 18 days to 15.5 years the dose of sodium benzoate from 250 to 375 mg/kg/d
- HHH syndrome: 19 patients, age from 1.5 years to 6 years, dose 250 mg mg/kg/d
- LPI: 3 patients?
- NAGS: 3 patients?

There is a wide range of both numbers of patients and duration of therapy for each of the listed condition. The CHMP acknowledged that this is due partly to the varying incidences of the listed enzyme deficiencies and severity of the disease state.

The data does appear to support the efficacy of oral sodium benzoate in the chronic therapy of CPS, OTC, ASS (Citrullinaemia), ASL (argininosuccinic aciduria), ARG1 (hyperargininaemia) and HHH;

although the data must be viewed with caution as it is of poor quality. It is not clear that formulation was oral in all cases, there appears to be a wide range of doses and the data is very limited in some of the listed enzyme deficiencies. The bridging of the proposed granule formulation and oral formulations in use in the studies is dependent on the satisfactory resolution of the PK questions raised.

With regards to the indication of NKH, the applicant has noted that the formulation of the drug was not reported in the majority of reports. As such the data to support this indication is not sufficient. The applicant should delete this indication or review other sources of data published or unpublished which they may wish to present to support the efficacy of oral therapy in NKH and the proposed posology.

The applicant was requested for an in-depth justification regarding the use of sodium benzoate in those enzyme deficiencies with a different pathological mechanism of hyperammonaemia such as LPI and in NAGSD; where the mode of action of sodium benzoate may render it ineffective. This has not been provided. It is noted that the Haberle 2012 publication excludes lysinuric protein intolerance in its proposed suggested guidelines. There are 3 patients with NAGSD briefly described in whom oral therapy with sodium benzoate was not used. There are no cases with LPI described. Further evidence of the efficacy of oral sodium benzoate in these conditions is required as the presented data is very limited **LoQ MO**.

It is noted that the survival rate of subjects with urea-cycle enzyme deficiencies improved significantly as compared to the historical data after implementation of new treatment regimens which consisted of nitrogen restriction (low protein diet) and supplementation with arginine and citrulline, and more recently administration of nitrogen scavenging medications such as sodium benzoate, sodium phenylacetate or sodium phenylbutyrate. Other treatment options included dialysis during acute episodes and liver transplantation. To what extent the observed improvement in the survival rate was related to nitrogen scavenging medications and specifically to the administration of sodium benzoate is not clear.

The applicant is applying for the treatment of urea cycle disorders with neonatal-onset presentation (complete enzyme deficiencies, presenting within the first 28 days of life). However, the product has to be sprinkled on to a spoonful of solid foods therefore it is not suitable for children younger than 6 months. For patients who cannot be fed orally with solid food the applicant proposed to use “dissolution protocol” as per recommendation included in section 6.6 of the SmPC. It is noted that in line with the proposed protocol the granules need to be grinded in a coffee grinder. It is very likely that this process will change the PK characteristic of the product. No data on the PK of the grinded granules (as well as on non-grinded granules) were provided by the applicant therefore clinical implications of such changes are unknown. In addition, no quality data were provided for the product to be mixed with baby milk or water. The applicant should further justify the proposed indication in this context **LoQ.**

In the presented studies regarding NKH, it was noted that many subjects were tube fed. As indicated in the SmPC Prohippur should not be administered by nasogastric tubes. The proposed protocol for dissolution and administration is not acceptable. The clinical implications of grinding the granules is unknown **LoQ**. Furthermore, there are quality concerns regarding the procedure and proposed administration via NG tube.

The applicant has failed to demonstrate acceptable palatability of the granules, this should be provided. As per guidelines evaluation of the patient acceptability of a paediatric preparation should be an integral part of the pharmaceutical and clinical development (EMA/CHMP/QWP/805880/2012 Rev. 2 Guideline on pharmaceutical development of medicines for paediatric use) **LoQ**.

The applicant states that since February 2017, the formulation of Prohippur is currently in use within a cohort ATU in France. Up to the time of writing a total of 25 patients (22 UCD, 3 NKH) have been requested the treatment under this program. No details of this study have been submitted **LoQ.**

There remains concern regarding the validity of the proposed posology and maximum doses, as discussed in the Clinical Pharmacology section above. **(LoQ MO)**

3.3.7. Conclusions on clinical efficacy

The bibliographic evidence presented in support of the current application lacks methodological rigor and therefore no reliable conclusion can be drawn on the basis of this evidence. The provided information on the methodology of the literature search is insufficient. There remains still uncertainly about the quality and completeness of evidence provided which the applicant should address. Evidence of critical appraisal and synthesis of the studies must be provided to permit evaluation of the quality of the data in line with the GCP requirements. applicant should also discuss the issue of selection bias.

It remains unclear from the information provided if oral sodium benzoate is efficacious in the chronic treatment of UCD and NKH. The duration of treatment, dose, and formulation of the study drug should clearly be identified for each indication. Clear information regarding how many subjects in the efficacy dataset were treated with oral sodium benzoate should be provided. The data are especially poor for the rarer enzyme deficiencies in UCD. There is a lack of discussion regarding the efficacy of sodium benzoate in n-acetylglutamate synthase deficiency NAGSD, lysinuric protein intolerance LPI, citrullinaemia type 1, hyperargininaemia, and ornithine translocase deficiency. Therefore, an in-depth justification and efficacy data is required regarding the use of sodium benzoate for these particular enzyme deficiencies, pertinently in those with a different pathological mechanism of hyperammonaemia such as LPI and in NAGSD where the mode of action of sodium benzoate may render it ineffective. Thus, major objections regrading clinical efficacy remain.

3.3.8. Clinical safety

The applicant performed a review of the literature. There are no systematic studies of sodium benzoate's adverse events. Most clinical safety data of sodium benzoate in the treatment of UCD or NKH have been acquired from cases reported in the literature. Since patients were not monitored under controlled, standardized conditions and were not provided with a diary for recording adverse events (AE), the reporting of these AE was not consistently documented. Moreover, AE may also be underreported as it can be difficult to distinguish between those of benzoate toxicity and those of hyperammonaemia since both may increase the uptake of tryptophan into the brain (Feillet 1998).

The adverse events reported in published clinical studies and individual case reports are listed and summarized in following table:

		Benzoate		AE Type	Frequency		
Reference	**N subjects (Indication)**	**Dose (mg/kg/d) route**	**Plasma conc (mmol/L)**		**N cases**	**Relative %**	**% total pop. (2556)**
Batshaw 1982	16 (UCD)	250 *po*	0.08±0.002	Vomiting	ns (2)	- 9 (Niemi 2006)	- (0.08)
				Elevated ASAT (77±16IU)	16	100	0.64
Green 1983	3 (UCD)	500 *iv*	2.14-16	Increase in free bilirubin	3	100	0.1
Brusilow 1984	20+13 (UCD)	250-500 *iv*	-	Increased anion gap	ns	-	-
							Nausea and vomiting after priming infusion
Simell 1986	5 (LPI)	288 *iv*	3-6	Dizziness	3	60	0.1
				Vomiting	2	40	0.08
Wolff 1986	ns (NKH)	900-1000	-	Anorexia vomiting renal tubular dysfunction	ns	-	-
Van Hove 1995	5 (NKH)	500 *iv & po*	-	Diarrhea	1	20	0.04
		750 *iv*	>8	Coma	1	20	0.04
		600-750 *iv & po*	-	Carnitine deficiency	2	40	0.08
Arnold 1997	2 (NKH)	500 *po*	-	Gastritis with GI bleeding	1	50	0.04
			750 *po*	Vomiting	1	50	0.04
				Seizures	1	50	0.04
				Carnitine deficiency	1	50	0.04
Hamosh 1998	4 (NKH)	500-750 *po*	-	Gastritis	ns	-	-
Chien 2004	5 (NKH)	250-500 *po*	-	GI intolerance	ns	-	-
Enns (*) 2007	332 (UCD)	250 *iv*	-	Vomiting	32	10	1.3
				Seizures	33	10	1.3
				Hypokalemia	32	10	1.3
Husson 2016	61 (UCD)	150-606 *iv*	-	Infusion line problems	18	30	0.70
				Neurologic complications	19	31	0.74
				Hepatocytolysis	12	20	0.47
				Liver failure	10	16	0.39
				Disseminated intravascular coagulation	4	7	0.16
				Acute renal failure	3	5	0.12
				Septic shock	1	2	0.04
				Left ventricular dysfunction	1	2	0.04
				Cardiorespiratory arrest	1	2	0.04
				Death	8	13	0.31

(*) Enns 2007, here only the AE with a relative high frequency (of ~10%) are listed

Patient exposure

None directly provided. However, the number of subjects either healthy volunteers (HV) or patients with UCD or NKH from the literature exposed - at least for some time - to sodium benzoate (even if considered as non-evaluable for absence of data) is detailed in the table below.

Exposure to sodium benzoate in HV, UCD, LPI, NKH, and HE subjects reported in the literature:

Type of study	Reference	Subjects		Exposure	
		Type	N	Duration	Daily dose (mg/kg) (except where indicated)
PK-PD	Snapper 1946	HV	9	-	5.8 g
	Wu 1961	HV	25	-	119-238
		RF	25		
		LF	9		
	Amsel 1969	HV	1	-	55
	Moolenar 1978	HV	10	-	4.2-7.0
	Mitch 1982	RF	7	5 d	10 g
	Green 1983	UCD	3	-	500
	Simell 1986	LPI	5	-	288
	Oyanagi 1987	UCD	2	-	130, 150
	Kubota 1988	HV	1	-	40, 80, 160
	Barshop 1989	UCD	2	-	180-650
		NKH	9		
	Kubota 1991	HV	6	-	40, 80, 160
	Feoli-Fonseca 1996	UCD	11	-	ns
	MacArthur 2004	HV	20	-	75-150
Clinical	Brusilow 1979	UCD	2	10 d	1.2 g, 6.25 g
	Brusilow 1980	UCD	1	11 d	160

	Batshaw 1980	UCD	7	< 2 d	250-500
	Batshaw 1982	UCD	26	7-62 mo	250-500
	Batshaw 1983	UCD	9	-	250
			1		800
	Brusilow 1984	UCD	7	< 4 d	250-500
	Msall 1984	UCD	26		250
	Call 1984	UCD	1	9 mo	410
	Guibaud 1984	UCD	1	30 mo	250-500
	Qureshi 1984	UCD	1	2 y	250-375
	Van de Bor 1984	UCD	1	15 mo	250
	Letartre 1985	UCD	1	3 y	250
	Batshaw 1988	UCD	1	8 y	200-375
	Maestri 1991	UCD	32	4.5 y mean	250
	Walter 1992	UCD	1	1.5 y	150-500
	Connelly 1993	UCD	2	5.8 y mean	ns
	Maestri 1995	UCD	24	5.6 y mean	250
	Renner 1995	UCD	1	5 y	200
	Maestri 1996	UCD	39	7.5 y max	250
	Uchino 1998	UCD	216	-	ns
	Bachmann 2003	UCD	44	-	ns
	Enns 2007	UCD	299	ns	250
	Al-Hassnan 2008	HHH	1	3 y	250
	De Groot 2010	UCD	2	3 wks max.	ns
	Grioni 2011	UCD	4	6.25 y mean	55-125
	Ko 2012	LPI	1	1 y	200
	Bergmann 2014	UCD	1	1 y	ns
	Boneh 2014	UCD	28	ns	ns
	Kalkan Uçar 2015	UCD	1	5.75 y	500
	Martin-Hernandez 2014	UCD	14	ns	165±80.5 to 184±92.3
	Rüegger 2014	UCD	77	ns	ns
	Martinelli 2015	HHH	26	ns	ns
	Blair 2015	UCD	1	1 year	ns
	Husson 2016	UCD	61	2 d median	150-606
	Unsinn 2016	UCD	45	ns	ns
	Ziter 1968	NKH	1	3 y	500
	Baumgartner 1969		1	7 mo	ns
	Trijbels 1974		1	3 y	100-300
	Krieger 1977		2	5 mo mean	225-500
	Von Wendt 1981		1	-	150-400
	Carson 1982		31 max	ns	ns
	Matalon 1983		2	1 y mean	125
	Cole 1985		3	ns	5 g/d
	Luder 1989		1	1 y	ns
	Hamosh 1992		1	1 y	500-750
	Zammarchi 1994		1	5 mo	500
	Van Hove 1995		5	3 y mean	500-750
	Alemzadeh 1996		1	10.5 mo	400
	Boneh 1996		6	2.8 y mean	250
	Arnold 1997		2	5.6 y mean	250-750

	Hamosh 1998		3	2.33 y mean	500-750
	Van Hove 1998		3	5 y mean	250-750
	Al-Essa 1999		1	3 wk	500
	Lu 1999		2	10 mo mean	250-300
	Maeda 2000		1	3 y	ns
	Neuberger 2000		1	26.5 mo	250-750
	Randak 2000		3	ns	500
	Van Hove 2000		3	2.1 y mean	480-500
	Wiltshire 2000		1	ns	250
	Korman 2002		1	17 mo	ns
	Aliefendioglu 2003		2	5.5 mo mean	250
	Chien 2004		5	3 y mean	250-500
	Hoover-Fong 2004		57	ns	ns
	Korman 2004		3	1.3 y mean	250
	Kure 2004		2	3.5 y mean	ns
	Roy 2004		3	ns	500
	Van Hove 2005		10	-	750
	Zenciroglu 2005		2	13 mo mean	ns
	Korman 2006		1	11 mo	250
	Chiong 2007		1	7 y	ns
	Demirel 2008		5	6.8 mo mean	250-750
	Mastrangelo 2008		1	11 y	250-500
	Rossi 2009		1	48 h	ns
	Yis 2009		1	1.5 y	750
	Suzuki 2010		1	9 mo	150
	Tsao 2005, 2010		2	6.25 y mean	600
	Dhamija 2011		1	4 mo	ns
	Hennermann 2012		38	ns	200-750
	Chiu 2015		1	1 mo	250-750
	Swanson 2015		113	ns	ns
	Bjoraker 2016		8	5.2 y mean	ns
	Misel 2013	HE	70 (in CT)	5 d - 6 mo	6.4-10 g
			>1000 (expert)	ns	4-10 g
Total			**2556**	2 d – 6 y mean	100-750

CT: clinical trial; d: day; h: hour; HE hepatic encephalopathy; HV healthy volunteers; LPI: lysinuric protein intolerance; mo: months; NKH: non ketotic hyperglycinemia; ns: not specified; UCD: urea cycle disorders; wk: week; y: years

According to the applicant 1389 HV/UCD/NKH subjects were identified in the literature. In addition, 27 cases of accidental overdoses were identified where the drug had been given in doses up to 4380 mg/kg/d (Enns 2007).

The applicant also notes that sodium benzoate has also been used in the management of overt hepatic encephalopathy (HE) and so the reports that refer to this have not been reviewed.

The number of oral administrations was 3-6 times per day in the chronic treatment of NKH patients (e.g. Van Hove 2005).

For the treatment of UCD, it appears that the posology varied with single doses usually administered as a bolus iv dose with benzoate/phenylacetate combination infused and repeated until the plasma ammonia levels normalized and before oral therapy with benzoate and other treatments (e.g. L-

arginine and restricted protein diet) was instituted on a long-term basis. Most subjects were exposed on a long-term therapy for over 6 months. The applicant considered it difficult to identify the number of UCD patients who after treatment of an acute episode have been treated in the long-term follow-up with benzoate.

In the indications targeted (UCD, NKH), the dose range spans from 100 mg/kg/d to 750 mg/kg/d in normal conditions of prescription or preparation. In UCD it rarely exceeds 500 mg/kg/d, whereas in NKH it is apparently recommended to not exceed 750 mg/kg/d. In adult patients, benzoate is generally given at a maximum dose of 10-12 g/day (or 5.5 g/sqm). Most subjects were exposed on a long-term therapy for over 6 months.

The shortest exposure to benzoate was during the acute treatment of hyperammonaemia episodes in patients with UCD or during pharmacodynamic evaluations e.g. in LPI (Simell 1986), usually during the acute treatment of ammonia decompensation episodes in UCD patients, often less than a couple of days or less. The longest exposure is centred around a mean of 6 or 7 years, with a maximum of 11 years reported once (Connelly 1993).

As indicated by the applicant the total number of patients (particularly those with UCD) exposed to sodium benzoate is extremely difficult to determine from the published literature.

1389 subjects with diagnosis of HV/UCD/NKH exposed to sodium benzoate were identified the literature however information provided on these subjects is limited especially in relation to the dose used, duration of exposure and method of administration (iv or oral administration).

The applicant does not specify the number of subjects exposed to the target dose and it is not clear how many subject received oral sodium benzoate. The relevant clarification needs to be provided for both UCD and NKH indications. The number of subjects exposed to sodium benzoate for the period longer than 6 months as well as 1 year should be specified. The applicant should provide a review and discussion of safety in long term use LoQ.

Adverse events

Treatment of UCD

In the treatment of UCD, oral sodium benzoate therapy has been associated with nausea and vomiting (Batshaw 1982, Simell 1986, Feillet 1998), the latter occurring in up to 9% of patients (Niemi 2006). Acute toxic AE such as vomiting and lethargy have always resolved within 12h of drug discontinuation and only occurred when plasma benzoate concentration exceeded 800 mg/L (Batshaw 1982). Anorexia and vomiting were also reported in healthy volunteers (HV) after boluses followed by continuous infusions of 11 g/m2/day (~1.65 mmol/kg/d) with the association product of benzoate and phenylacetate (MacArthur 2004). Brain increases in tryptophan uptake and serotonin turnover have been reported in hyperammonaemia, therefore the association of anorexia/food refusal with increased serotonin turnover in children with UCD.

Other neurological symptoms (tinnitus, vertigo, headache and visual disturbance) have also been recorded (Misel 2013).

Gastric irritation is a well-known dose-dependent AE of sodium benzoate (Van Hove 2005). It was reported in one case as leading to gastric bleeding related to hyperosmolarity (587 mOsm/L) of the formulation used (Arnold 1997).

Most oral benzoate preparations have a bitter and extremely unpleasant salty taste with a burning sensation upon swallowing (Misel 2013). Due to its unpalatability, long-term compliance to oral benzoate treatment may be poor or the initiation of treatment may be difficult. Oral sodium benzoate

has sometimes been administered via gastrostomy/nasogastric tubes or the taste masked by mixing it with sucrose powder in a double-blind placebo-controlled study over a period of 4 weeks (Wiltshire 2000) or with dextrose (Misel 2013). Sodium benzoate has been compounded into 500-mg capsules which can help mask the unpleasant taste but remain problematic in patients who cannot swallow. Moreover, the capsules should not be opened unless appropriate contact and airborne precautions are followed.

In a retrospective study of UCD patients treated with the iv combination product of sodium benzoate and sodium phenylacetate, AE were reported in just over 50% of patients, however most were likely related to the underlying primary disease or the patient's clinical status (Enns 2007).

AE on treatment with sodium benzoate in UCD, LPI, NKH, HE and HV subjects

Reference	N subjects (Indication)	Benzoate		AE			
		Dose (mg/kg/d) *route*	Plasma concentration (mmol/L)	Type	Frequency		
					N cases	Relative %	% total pop. (2556)
Batshaw 1982	16 (UCD)	250 *po*	0.08±0.002	Vomiting	ns (2)	- 9 (Niemi 2006)	- (0.08)
				Elevated ASAT (77±16IU)	16	100	0.64
Green 1983	3 (UCD)	500 *iv*	2.14-16	Increase in free bilirubin	3	100	0.1
Brusilow 1984	20+13 (UCD)	250-500 *iv*	-	Increased anion gap	ns	-	-
				Nausea and vomiting after priming infusion			
Simell 1986	5 (LPI)	288 *iv*	3-6	Dizziness	3	60	0.1
				Vomiting	2	40	0.08
Wolff 1986	ns (NKH)	900-1000	-	Anorexia vomiting renal tubular dysfunction	ns	-	-
Sushma 1992	38 (HE)	10 g/d x 7.9 d *po*	-	Nausea	15	39	0.6
				Vomiting	10	26	0.4
				Epigastric discomfort	10	26	0.4
Van Hove 1995	5 (NKH)	500 *iv & po*	-	Diarrhea	1	20	0.04
		750 *iv*	>8	Coma	1	20	0.04
		600-750 *iv & po*	-	Carnitine deficiency	2	40	0.08
Arnold 1997	2 (NKH)	500 *po*	-	Gastritis with GI bleeding	1	50	0.04
		750 *po*		Vomiting	1	50	0.04
				Seizures	1	50	0.04
				Carnitine deficiency	1	50	0.04
Hamosh 1998	4 (NKH)	500-750 *po*	-	Gastritis	ns	-	-
Chien 2004	5 (NKH)	250-500 *po*	-	GI intolerance	ns	-	-
MacArthur 2004	3 (HV)	11g/m²/d *iv*	3.4±0.3	Nausea, vomiting	3	100	0.1
				Somnolence	3	100	0.1
Enns (*) 2007	332 (UCD)	250 *iv*	-	Vomiting	32	10	1.3
				Seizures	33	10	1.3
				Hypokalemia	32	10	1.3
Husson 2016	61 (UCD)	150-606 *iv*	-	Infusion line problems	18	30	0.70
				Neurologic complications	19	31	0.74
				Hepatocytolysis	12	20	0.47
				Liver failure	10	16	0.39
				Disseminated intravascular coagulation	4	7	0.16
				Acute renal failure	3	5	0.12

Not many reports of adverse events associated with the benzoate treatment were identified in the literature. This could indicate that benzoate was tolerated well by most patients however taking into consideration that the safety was not systematically studied the final conclusion cannot be made.

Gastrointestinal adverse events were reported in subjects receiving sodium benzoate. It seems that the most commonly reported gastrointestinal adverse events were nausea and vomiting.

However serious and potentially life-threatening gastrointestinal adverse events were also reported including gastritis and GI bleeding. Potential risk of GI bleeding should be further discussed by the applicant **LoQ.**

As indicated by the applicant gastrointestinal symptoms could be minimized by using co-prescribe H2-receptor antagonists or proton pump inhibitors. The applicant is requested to further investigate this recommendation. If justified the relevant information could be included in the product information **LoQ**

Serious adverse events and deaths

Serious but rare adverse events that have been reported include metabolic acidosis although with no further details were provided (Misel 2013). Cases of overdoses (27 reported so far in the literature) should be considered as serious AE and they are therefore detailed and summarized in the following table

Published and not reported (NR) cases of sodium benzoate overdoses

Reference (Number of cases)	Benzoate				Symptoms
	Indication	Drug	Dose (mg/kg/d)	Plasma concentration (mmol/L)	
Batshaw 1982 (2)	UCD	PA+BA *iv*	800	8.54	Vomiting, irritability
			-	-	Vomiting, irritability
Brusilow 1984 (1)			2500	-	Death
Praphanphoj 2000 (3)			915 over 12h	10.6	Confusion (1)-coma (2), Kussmaul respiration, metabolic acidosis (3), cerebral oedema (2) death (2)
			1750 over 18h	-	
			750 over 10h	5.58	
Van Hove 2005 (1)	NKH	BA *po or iv*	2.5 higher than required	15.4	Kussmaul respiration (1), metabolic acidosis (1) shock with coma (1)
Enns 2007 (17)	UCD	PA+BA *iv*	370-4380	-	including 13 deaths no information on the 5 other cases
Hennermann 2016 (NR 2)	NKH	BA *po*	ns	-	2 deaths
Husson 2016 (1)	UCD	BA *iv*	606	-	Neurological decompensation, seizures, hyperthermia, death (1)
Total 27					

BA benzoate; PA phenylacetate; NR not reported

One patient was inadvertently administered the volume of benzoate as a 25% solution instead of a 10% solution. Eighteen hours after four applications, the patient was in metabolic acidosis with coma, vomiting and Kussmaul breathing requiring intubation and ventilation. She had severe hypernatremia (154.4 mmol/L), hypokalaemia (2.16 mmol/L), a large anion gap (32.8 mmol/L), hypocalcaemia (1.5 mmol/L), and mild hyperammonaemia (75 µmol/L), transaminases were normal. Glycine was reduced to 62 µmol/L (normal range 100–350). Benzoate plasma level was 15.4 mmol/L (therapeutic level < 5mmol/L as per Van Hove 1995). She was stabilized by discontinuation of benzoate and substitution of glycine, potassium and calcium. She received conventional haemodialysis. Immediately after starting dialysis, benzoate plasma level dropped to less than 0.1 mmol/L. The next morning the patient recovered and previous therapy was reinstituted.

After inadvertent overdoses or dose increases of an intravenously applied combination of benzoate and phenylacetate, cases of hyperammonaemia with a clinical toxicity symptomatology mimicking hyperammonaemia with anorexia, increased seizures, irritability, lethargy, and coma have been

reported (Batshaw 1982, Brusilow 1984, Batshaw 1988, Van Hove 1995, Praphanphoj 2000, Husson 2016).

In addition, thirteen cases of death are reported in 17 cases of overdoses (370-4380 mg/kg/d), including two massive ones (9 and 17 times of the recommended dose of sodium phenylacetate and sodium benzoate), in a retrospective open-label 25-year-observationnal study of patients with UCD (Enns 2007).

Deaths

Over 2558 subjects having received sodium benzoate, the death rate in these very serious diseases reaches 3.9% (102 cases). Their relationship with the treatment is almost never available, although in a recent retrospective survey, no death over the 8 that occurred was attributed to sodium benzoate (Husson 2016). In the only randomized comparative trial listed, the death rate is similar in the sodium benzoate's arm to that in the comparator's arm (Sushma 1992). Therefore, except for the cases of overdose described above, it is not possible to attribute these deaths to the treatment with sodium benzoate or the underlying disease.

Laboratory findings

Benzoate has in theory a biphasic effect on plasma ammonia, at least in animals (Batshaw 1988). At doses leading to benzoate accumulation with little hippurate excretion, benzoate acts by inhibiting the urea cycle via diverting N-acetylglutamate (Tremblay 1993, Van Hove 1995). However, this remains an experimental finding (Tremblay 1993). This would represent a paradoxical effect of benzoate on ammonium levels, i.e. potentiating hyperammonaemia. At these doses, the intermediate, benzoyl CoA, accumulates and CoA-dependent processes (amongst which is the formation of N-Acetyl Glutamate, the activator of the first enzyme in the urea cycle, carbamyl phosphate synthetase) are consequently impaired, resulting in inhibition of the urea synthetic activity. Conversely, at doses that results in a significant alternate pathway of waste nitrogen excretion as hippurate, benzoate alleviates hyperammonaemia, and its CoA-inhibitory effect is counterbalanced. Accentuation of hyperammonaemia should not occur at the benzoate doses recommended for treatment of UCD (Batshaw 1988). Hyperammonaemia was recorded in 18 cases out of 332 UCD patients having received the iv combination product of sodium phenylacetate and sodium benzoate, this AE as others may possibly be related to the underlying disorder or to the patient's clinical status (Enns 2007).

Hypokalaemia was one of the most frequently reported AE in the 25-year retrospective study of the iv combination product in UCD patients with 32 cases reported, as compared to 22 cases of acidosis (Enns 2007). Hyperkalaemia in the context of multiorgan failure with ventricular tachycardia was reported in one case (Husson 2016). Electrolyte imbalance such as hypervolemic hyponatremia with worsening ascites is possible, although conversely sodium benzoate may cause hypernatremia, and the sodium content of sodium benzoate must be considered into account in the authorized dietary sodium intake (Misel 2013).

An increased anion gap may be noted during intravenous therapy using the mixture containing benzoate and phenylacetate because of their respective accumulation in plasma together with that of their respective conjugation products, the sum of which may attain levels of 7 mM (Brusilow 1984). In 3 cases of overdoses retrospectively detailed, the maximal increased anion gap levels reported were 32, 52, and 39 mEq/L, respectively (Praphanphoj 2000). Metabolic acidosis has also been reported following iv sodium benzoate not overdosed (Husson 2016).

Carnitine deficiency which has been reported with high doses of sodium benzoate - as benzoate binds to carnitine to form benzoyl-carnitine (Van Hove 1995, Arnold 1997) - is usually prevented by carnitine

supplementation, however carnitine levels should be monitored regularly (Van Hove 1995, Feuillet 1998).

Renal tubular dysfunction syndrome with electrolyte abnormalities (metabolic acidosis, hypokalaemia, glycosuria, hypocalcaemia) were also reported with benzoate doses of 900–1000 mg/kg/day (Tremblay 1993, Van Hove 1995). Therefore, in patients with chronic kidney disease long-term therapy with benzoate requires careful evaluation of a potential toxicity of hippurate which prolonged elevation in plasma might saturate renal tubular secretory processes, benzoate's direct toxicity being theoretically unlikely because of its fast conversion into hippurate (Mitch 1982).

The potential increase in free bilirubin in neonates should be considered (Green 2003) however no significant hyperbilirubinemia or kernicterus have ever been attributed to benzoate treatment (Niemi 2006).

Safety in special populations

The effect of cirrhosis and/or kidney disease in the generation and elimination of hippurate is not well known. After conjugation of benzoate to glycine, hippurate is cleared by the kidney at fivefold the glomerular filtration rate (Batshaw 1994). The pharmacokinetics of benzoate was studied in patients with renal impairment and benzoate does not accumulate in CRF patients (Mitch 1982). However, as compared to healthy volunteers, mean excretion constant for exogenous hippurate was lower in patients with chronic renal failure (Wu 1961).

Dialysis has been shown to increase excretion of hippurate from about 20% in controls to almost 50% in dialysed patients (Misel 2013).

Unlike patients with inborn errors of metabolism, the ability of benzoate to remove nitrogen is theoretically limited in situations in which liver conjugation is impaired. In 9 patients with liver disease presented above (Wu 1961), the values for the hippurate synthesis constant were comparable to values in the healthy control subjects. The conversion to hippurate was relatively slower which suggests that in patients with liver failure the capacity of the metabolic pathway for benzoate could be slightly reduced.

Safety related to drug-drug interactions and other interactions

Although salicylates can impair mitochondrial function in the liver, there was no apparent effect on the elimination of hippurate from the administration of 2-3 g of salicylic acid 2 h before benzoic acid. Salicylate had no measurable effect on the proportion of hippuric acid and benzoyl glucuronide excreted in the urine after benzoate administration, and on the excretion rate constant of hippuric acid. Conversely, benzoate had a marked, rapid, short dose-dependent inhibitory effect on salicylurate formation and it was shown that excretion of salicyluric acid which had reached a plateau at about 1 h was immediately decreased after benzoate administration, and increased again to return to the plateau level in ~5 h (Amsel 1969).

Penicillin competes with hippurate for active secretion by renal tubules, which may affect the overall disposition of sodium benzoate. Probenecid is also known to inhibit the renal transport of many organic compounds, including aminohippuric acid, and may affect renal excretion of hippurate (Misel 2013).

Benzoate interferes with carnitine and may promote carnitine deficiency therefore supplementation with carnitine may be necessary to avoid induction of carnitine deficiency during benzoate therapy (Hennermann 2012).

Monitoring of plasma ammonium, glycine, benzoate, carnitine and hippurate levels is advised. To achieve the best possible results, therapeutic monitoring should include measurements of plasma ammonia and amino acids. The aim is to reduce ammonia concentration below twice the upper normal limit in UCD, and to keep plasma glycine concentration in the normal concentration range in NKH (< 250 µmol/L) then the clinical objective of treatment is the reduction in seizures, increase of alertness and the reduction in the numbers of anticonvulsants.

Discontinuation due to AES

It has not been practically possible to identify from the literature cases which patients discontinued the treatment during studies published, apart for the patients who discontinued from death or overdose.

Discussion on clinical safety

501 UCD patients out of 544 who were receiving a dose of 250-500 mg/kg/d. A total of 118 were treated for more than 6 months, and 114 more than a year. Similarly, from the published cases, there was 96 NKH patients out of 135 who were receiving a dose of 300-750 mg/kg/d. A total of 72 were treated for more than 6 months, and 61 more than a year.

The applicant has stated that “recurrent esophagitis and gastritis are apparently frequently observed AE”. Furthermore, the applicant states this AE occurs if “benzoate preparation is not sufficiently diluted”. There is no information presented as to the incidence of this AE, whether this AE is due to local irritation or what is the mechanism of action for the adverse event. This is of concern as there no assurance that the granule formulation currently presented will behave in a similar fashion to other oral formulations or indeed will be associated with an increased incidence of adverse events **LoQ.**

Serious and life-threatening adverse events were reported in patients receiving sodium benzoate including GI bleeding (2 cases), liver failure (10 cases+ 12 cases of liver cytolysis without liver failure), disseminated intravascular coagulation (5 cases), acute renal failure (3 cases), left ventricular dysfunction (1 case), cardiorespiratory arrest(1 case), seizures (36? cases), metabolic acidosis with coma (4 cases in case of overdose). A significant number of cases of liver failure, DIC and seizures is worrisome. As the analysis made by the applicant is based on the review of the literature cases it is difficult to establish relatedness of these adverse events to sodium benzoate. Further discussion in relation to information which needs to be included in section 4.8 of the SmPc is required **LoQ.**

As indicated by the applicant, hypokalaemia was one of the most frequently reported laboratory finding in the 25-year retrospective study of iv sodium benzoate in UCD patients. However it is noted that hypokalemia is not listed in the SmPc. Other laboratory abnormalities reported include acidosis, hyponatremia. Sodium benzoate may cause hypernatremia due to its sodium content of sodium benzoate must be considered into account in the authorized dietary sodium intake.

It needs to be highlighted that benzoate treatment has a narrow, patient-dependent, therapeutic window, especially in subjects with NKH. If the amount of benzoate exceeds the availability of glycine, and glycine levels are below normal a significant toxicity may develop including development of metabolic acidosis with coma. The applicant recognizes this issue by adding the following recommendation to section 4.2 of the SmPC: “To prevent toxicity, the dose of benzoate should then be gradually increased, e.g. by 50 mg/kg/day, and plasma glycine and benzoate levels should be monitored before any subsequent dose change. The maximum daily dose should be less than 750 mg/kg/day.” The applicant should further discuss this significant safety issue. The relevant justification for the proposed risk minimization measures and SmPc wording should be provided. In addition, it is considered that information provided in the SmPC is insufficient. The acceptable glycine and benzoate

levels are not provided. Also, it should be specified how frequently glycine and benzoate levels should be checked, in what period after administration of sodium benzoate **LoQ.**

The requirement for validated assays of sodium benzoate measurement appears to be essential for the safe use of this drug. Especially in the neonatal period in UCD, where induction of hippurate synthesis may be delayed because of an immature N-acetylase and plasma benzoate concentrations may reach potentially toxic levels. Adequate assurance that these validated assays are available in the EU has not been provided **LoQ.**

The applicant has not provided any information on the potential differences in the safety profile of sodium benzoate in neonates as compared to older children and young adults. However, such differences are likely to occur taking into the consideration immaturity of enzymes responsible for the acylation of benzoate with CoA. As discussed above a significant variability in the metabolism of sodium benzoate was observed in children and it cannot be concluded based in the provided data that the proposed doses of sodium benzoate are safe in this patient population **LoQ.**

The applicant was asked to describe the methodology used to identify ADRs and justify the wording proposed for section 4.8of the SmPC. The applicant stated that all AEs identified in the literature were included in the proposed SmPC and no further justification of the proposed wording was provided, which is not acceptable The applicant included in the SmPC frequencies of the reported adverse reactions however the justification for these frequencies has not been provided. It is noted that some AEs reported in the literature are included in the SmPC and some AEs are not included and the reason for this is not clear **LoQ.**

3.3.9. Conclusions on clinical safety

The current presented information on safety is poor and precludes the making of a conclusion on clinical safety.

3.4. Risk management plan

Safety concerns

The applicant proposed the following summary of safety concerns in the RMP:

Table Summary of the Safety Concerns

Summary of safety concerns	
Important identified risks	1. Coma 2. Seizures 3. Renal tubular dysfunction 4. Gastritis, gastric bleeding 5. Anorexia 6. Decreased blood carnitine 7. Increased blood free bilirubin 8. Increased blood transaminases
Important potential risks	9. Use in patients with hepatic insufficiency

Summary of safety concerns	
	10. Use in patients with renal insufficiency 11. Acute hyperammonaemic encephalopathy 12. Use in patients with congestive heart failure and clinical conditions involving sodium retention with oedema and in patients on controlled sodium diet 13. Use in pregnancy 14. Overdose
15. Missing information	16. Use during breast feeding

Having considered the data in the safety specification, the following issues should be addressed:

- Long-term safety should also be listed as a missing information point in the RMP.
- Reference to "phenylacetate" in section SII, table 2 of the RMP should be reviewed and corrected as necessary.
- The list of safety concerns does not adequately reflect the lack of information on potential drug-drug interactions with Prohippur and this should be addressed.
- Use in patients with hepatic or renal insufficiency and also use in pregnancy cannot be considered as potential risks unless there is a specific safety concern in those populations which would have to be detailed in the RMP. The applicant should either detail the specific safety concerns or delete 'Decreased blood carnitine', 'Increased blood free bilirubin', 'Increased blood transaminases' from the safety specification, and to have 'Use in patients with hepatic insufficiency', 'Use in patients with renal insufficiency', and 'Use in pregnancy' listed as missing information.
- As regards the compliance to the format of the RMP (see EMA/465932/2013 rev 2), it is noted that the section "SVII.3 Details of important identified and potential risks from clinical development and post-authorisation experience (including newly identified)" of the submitted RMP is not in line with the requirements of the relevant guidelines. The applicant is therefore requested to redraft the section with all the fields requested from the template, for each identified and potential risk.

Pharmacovigilance plan

In consideration that the treatment is proposed for the chronic management of the disease, further data on the long-term administration should be collected. Pending the CHMP opinion on the proposed indications, the applicant is requested to consider to implement a registry study to collect relevant long-term safety and efficacy data in patients with UCDs focusing, above other, on safety information in patients with renal/hepatic impairment, on pregnancy outcomes in children born to female patients exposed to PROHIPPUR during pregnancy, on children exposed during lactation.

Also for the indication in NKH population, in case of CHMP positive opinion, a suitable proposal for the collection of data in long-term clinical practice is expected.

The studies should be classified as category 3 and the relevant sections and tables of RMP should be updated accordingly, including a brief description of source, objectives and timelines.

Risk minimisation measures

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Coma	Appropriate wording that is relevant to minimizing the risk is detailed in the Summary of Product Characteristics (SPC) and Package Leaflet (PL)	None
Seizures	Appropriate wording that is relevant to minimizing the risk is detailed in the Summary of Product Characteristics (SPC) and Package Leaflet (PL)	None
Renal tubular dysfunction	Appropriate wording that is relevant to minimizing the risk is detailed in the Summary of Product Characteristics (SPC) and Package Leaflet (PL)	None
Gastritis, gastric bleeding	Appropriate wording that is relevant to minimizing the risk is detailed in the Summary of Product Characteristics (SPC) and Package Leaflet (PL)	None
Anorexia	Appropriate wording that is relevant to minimizing the risk is detailed in the Summary of Product Characteristics (SPC) and Package Leaflet (PL)	None
Decreased blood carnitine	Appropriate wording that is relevant to minimizing the risk is detailed in the Summary of Product Characteristics (SPC) and Package Leaflet (PL)	None
Increased blood free bilirubin	Appropriate wording that is relevant to minimizing the risk is detailed in the Summary of Product Characteristics (SPC) and Package Leaflet (PL)	None
Increased blood transaminases	Appropriate wording that is relevant to minimizing the risk is detailed in the Summary of	None

	Product Characteristics (SPC) and Package Leaflet (PL)	
Use in patients with hepatic insufficiency	Appropriate wording that is relevant to minimizing the risk is detailed in the Summary of Product Characteristics (SPC) and Package Leaflet (PL)	None
Use in patients with renal insufficiency	Appropriate wording that is relevant to minimizing the risk is detailed in the Summary of Product Characteristics (SPC) and Package Leaflet (PL)	None
Acute hyperammonaemic encephalopathy	Appropriate wording that is relevant to minimizing the risk is detailed in the Summary of Product Characteristics (SPC) and Package Leaflet (PL)	None
Use in patients with congestive heart failure and clinical conditions involving sodium retention with oedema and in patients on controlled sodium diet	Appropriate wording that is relevant to minimizing the risk is detailed in the Summary of Product Characteristics (SPC) and Package Leaflet (PL)	None
Use in pregnancy	Appropriate wording that is relevant to minimizing the risk is detailed in the Summary of Product Characteristics (SPC) and Package Leaflet (PL)	None
Overdose	Appropriate wording that is relevant to minimizing the risk is detailed in the Summary of Product Characteristics (SPC) and Package Leaflet (PL)	None
Use during breast feeding	Appropriate wording that is relevant to minimizing the risk is detailed in the Summary of Product Characteristics (SPC) and Package Leaflet (PL)	None

The applicant proposed only routine RMMs.

Given the risk of medication error and toxic overdose the applicant should discuss if appropriate risk minimisation (included aRMM) are needed.

Conclusion

The RMP Part III-VI could be acceptable provided an updated RMP and satisfactory responses to the list of questions in section 6.3.

Public summary of the RMP

The public summary of the RMP does require revision.

4. Orphan medicinal products

Orphan designation

Prohippur has been designated as an orphan drug in the EU (on 14th July 2016) for carbamoyl-synthase-1 deficiency, citrullinaemia type 1, hyperargininaemia, ornithine transcarbamylase deficiency, on 29th August 2016 for lysinuric protein intolerance for ornithine translocase deficiency and on 18th November 2016 for N-acetylglutamate synthase deficiency, argininosuccinic aciduria and non-ketotic hyperglycinaemia (via transfer of designation)

According to the conclusion of the COMP (Opinion dated 5 September 2016 EMA/COMP/450237/2016) the prevalence of ornithine transcarbamylase deficiency is less than 0.1 in 10,000 individuals in the EU.

According to the conclusion of the COMP (Opinion dated 13 December 2016 EMA/683957/2016) the prevalence of argininosuccinic aciduria is less than 0.1 in 10,000 individuals in the EU.

According to the conclusion of the COMP (Opinion dated 13 December 2016 EMA/683954/2016) the prevalence of NAGS deficiency affected less than 0.01 in 10,000 people in the European Union (EU).

According to the conclusion of the COMP (Opinion dated 22 September 2016 EMA/COMP/498293/2016) the prevalence of ornithine translocase deficiency affected less than 0.1 in 10,000 people in the European Union (EU).

According to the conclusion of the COMP (Opinion dated 22 September 2016 EMA/COMP/494165/2016) the prevalence of lysinuric protein intolerance affected approximately 0.5 in 10,000 people in the European Union (EU).

According to the conclusion of the COMP (Opinion dated 5 September 2016 EMA/COMP/450251/2016) the prevalence of hyperargininaemia affected less than 0.01 in 10,000 people in the European Union (EU).

According to the conclusion of the COMP (Opinion dated 5 September 2016 EMA/COMP/450240/2016) the prevalence of citrullinaemia type 1 affected less than 0.1 in 10,000 people in the European Union (EU).

Similarity

The application contained a critical report pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, addressing the possible similarity with authorised orphan medicinal products.

The assessment of similarity is appended to this report.

5. Benefit risk assessment

5.1.1. Disease or condition

Prohippur is indicated as adjunctive therapy in the chronic management of non ketotic hyperglycinemia, as well as that of urea cycle disorders including carbamoyl-phosphate synthase-1 deficiency, ornithine transcarbamylase deficiency, citrullinaemia type 1, argininosuccinic aciduria, hyperargininaemia, n-acetylglutamate synthase deficiency, ornithine translocase deficiency and lysinuric protein intolerance.

The proposed indication is in all urea cycle disorders patients with neonatal-onset presentation (complete enzyme deficiencies, presenting within the first 28 days of life). It is also indicated in patients with late-onset disease (partial enzyme deficiencies, presenting after the first month of life) who have a history of hyperammonaemic encephalopathy.

Urea Cycle Disorder (UCD) represents a deficiency of any one of six enzymes which are responsible for removing ammonia from the bloodstream by converting it into urea (e.g., carbamyl phosphate synthetase (CPS), N-acetylglutamate synthetase (NAGS), ornithine transcarbamylase (OTC), argininosuccinic acid synthetase (AS), argininosuccinate lyase (AL/ASA), arginase (AG)) (Maloney 2010). Ammonia itself is produced as an intermediate from amino acid catabolism. An accumulation of glutamine and alanine has been shown for all subtypes of UCD (Leonard 2002). The main treatment in UCD focuses on the alternative excretion pathway for waste nitrogen in form of ammonia by compounds that are conjugated to respective precursor amino acids (e.g., glycine) which are terminally excreted.

Non Ketotic Hyperglycinaemia (NKH) also known as glycine encephalopathy is an autosomal recessive disorder of the glycine metabolism. Patients are characterized by abnormally high glycine concentrations accumulating in plasma and cerebrospinal fluid (CSF), which is considered to be the main cause of toxicity (Beyoglu 2012).

In both indications, NKH and UCD, sodium benzoate acts as a nitrogen scavenger, binding nitrogen in form of glycine (NKH and UCD) or ammonia (UCD), respectively, followed by urinary excretion (Arnstein 1951; Bridges 1970; Barshop 1989; Beyoglu 2012).

5.1.2. Available therapies and unmet medical need

The applicant states there is no authorized oral presentation of sodium benzoate available in the European market. The present bibliographic application is for the registration of a new formulation (LUC-08/Prohippur®) of a pellet presentation of sodium benzoate in the chronic oral treatment of UCD and of NKH.

Ravicti (EMEA/H/C/003822) authorised in 2015, is an oral liquid formulation of glycerol phenylbutyrate which is indicated for use as adjunctive therapy for chronic management of adult and paediatric patients ≥2 months of age with urea cycle disorders (UCDs) including deficiencies of carbamoyl phosphate-synthase-I (CPS), ornithine carbamoyltransferase (OTC), argininosuccinate synthetase (ASS), argininosuccinate lyase (ASL), arginase I (ARG) and ornithine translocase deficiency hyperornithinaemia-hyperammonaemia homocitrullinuria syndrome (HHH) who cannot be managed by dietary protein restriction and/or amino acid supplementation alone.

Pheburane, EMEA/H/C/002500, authorised 2013, sodium phenylbutyrate, 483mg/g granules, is indicated as adjunctive therapy in the chronic management of urea cycle disorders, involving deficiencies of carbamylphosphate synthetase, ornithine transcarbamylase or argininosuccinate synthetase. It is indicated in all patients with neonatal-onset presentation (complete enzyme deficiencies, presenting within the first 28 days of life). It is also indicated in patients with late-onset disease (partial enzyme deficiencies, presenting after the first month of life) who have a history of hyperammonaemic encephalopathy.

Pheburane is a hybrid of Ammonaps EMEA/H/C/000219, active substance sodium phenylbutyrate, which has been authorised in the EU since 1999. Ammonaps is indicated as adjunctive therapy in the chronic management of urea cycle disorders, involving deficiencies of carbamylphosphate synthetase, ornithine transcarbamylase or argininosuccinate synthetase.

5.1.3. Main clinical studies

This marketing authorisation application has been submitted under Article 10a and thus no clinical studies have been conducted. The applicant states that a placebo-controlled clinical trial of benzoate has not been performed because of the risks of death or severe brain-damaging episodes of hyperammonaemia in untreated children. The studies presented as evidence for efficacy in the application include the use of different drugs and protocols given at different dosages at different ages, in different years, in over 80 medical institutions with varying resources. None of the presented studies were conducted as placebo-controlled clinical trials of benzoate.

The bibliographic evidence presented has many limitations in terms of demonstrating efficacy. When an application is made relying wholly on bibliographic evidence, two crucial criteria must be considered: completeness of the evidence and quality of studies. Thus, no conclusion can be made regarding the efficacy of sodium benzoate in the proposed indications of NKH and early and late onset UCD; at the proposed posology and method of administration, for the following reasons: the search criteria have not been provided. Therefore, it is difficult to assess whether the evidence presented is complete. Evidence of critical appraisal and synthesis of the studies must be provided to permit evaluation of the quality of the data in line with the GCP requirements.

It is presently unclear from the information provided if sodium benzoate is efficacious in the treatment of UCD and NKH.

5.2. Favourable effects

In both indications, NKH and UCD, sodium benzoate appears to act as a nitrogen scavenger, binding nitrogen in form of glycine (NKH and UCD) or ammonia (UCD), respectively, followed by urinary excretion (Arnstein 1951; Bridges 1970; Barshop 1989; Beyoglu 2012). Sodium benzoate is converted by acylation in the liver into its coenzyme A (CoA) ester, benzoyl-CoA. The latter compound is conjugated to glycine to form hippurate, which is excreted by the kidney. Hippurate contains 1 waste nitrogen atom, so 1 mole of nitrogen is removed for each mole of sodium benzoate administered (Batshaw 1983). In the presence of large amounts of benzoate and glycine, the level of free CoA is a rate limiting factor in the formation of benzoyl-CoA, itself a rate-limiting factor for glycine-N-acylase (Gregus 1992). The increased conjugation of glycine with benzoate tends to concomitantly reduce the glycine pool (Van Hove 1995). Thus, the mechanism of action of sodium benzoate with regards to excretion of nitrogen and thus clearance of ammonia and glycine is via its metabolite - hippurate. To reduce the risk of toxicity due to accumulation, the plasma levels of sodium benzoate must be measured during therapy and on dose escalation. The presented studies suggest the PD effects of sodium benzoate in the proposed patient population, with studies demonstrating reducing ammonia

levels in UCD and glycine levels in NKH. However, significant variability in the response was observed and the amounts of removed ammonia was lower than theoretically predicted.

The survival rate of subjects with urea-cycle enzyme deficiencies improved significantly as compared to the historical data after implementation of new treatment regimens including nitrogen scavenging medications however as the efficacy was not systematically studied a true effect of sodium benzoate is unknown. Also the frequency of hyperamonemic episodes in patients receiving nitrogen scavenging medications decreased however patients were receiving other treatments as well.

The data presented by the applicant indicate that in the majority of cases after administration of benzoate the level of glycine in plasma decreased in patients with Non Ketotic Hyperglycinaemia. Benzoic acid is activated to benzoyl-CoA, then is conjugated with glycine to form hippurate, which is excreted in the urine, thus, eliminating glycine and reducing glycine levels. In many of subjects normalization of the glycine level was achieved. The level of glycine in cerebrospinal fluid (CSF) decreased however normalization of glycine concentration in CSF was not achieved.

The provided data indicates that benzoate may have some role in a reduction of seizures and/or myoclonic jerks in patients with NKH. Some link between the dose of sodium benzoate and seizure control could be noted. However, as the effect on seizure control was not systematically studied and patients were also receiving other treatments including dextromethorphan and anticonvulsants a true role of benzoate is difficult to determine

5.3. Uncertainties and limitations about favourable effects

Generally, there remains inadequate data and deficiencies in reporting and discussion of the available PK data regarding absorption, distribution, metabolism and excretion. In addition, adequate data to support the exposure of Prohippur in the proposed population has not been provided. Inadequate information is provided by the applicant to bridge the proposed formulation (a granules formulation) to the various formulations described in literature, therefore, it cannot be concluded that Prohippur has the same PK as these formulations. Therefore, it is difficult to assess whether the proposed posology is either efficacious or safe.

The bibliographic evidence presented in support of the current application lacks methodological rigor and therefore no reliable conclusion can be drawn on the basis of this evidence. The provided information on the methodology of the literature search is insufficient. There remains still uncertainly about the quality and completeness of evidence provided which the applicant should address. Evidence of critical appraisal and synthesis of the studies must be provided to permit evaluation of the quality of the data in line with the GCP requirements. applicant should also discuss the issue of selection bias.

It remains unclear from the information provided if oral sodium benzoate is efficacious in the chronic treatment of UCD and NKH. The duration of treatment, dose, and formulation of the study drug should clearly be identified for each indication. Clear information regarding how many subjects in the efficacy dataset were treated with oral sodium benzoate should be provided. The data are especially poor for the rarer enzyme deficiencies in UCD. There is a lack of discussion regarding the efficacy of sodium benzoate in n-acetylglutamate synthase deficiency NAGSD, lysinuric protein intolerance LPI, citrullinaemia type 1, hyperargininaemia, and ornithine translocase deficiency. Therefore, an in-depth justification and efficacy data is required regarding the use of sodium benzoate for these particular enzyme deficiencies, pertinently in those with a different pathological mechanism of hyperammonaemia such as LPI and in NAGSD where the mode of action of sodium benzoate may render it ineffective.

5.4. Unfavourable effects

501 UCD patients out of 544 who were receiving a dose of 250-500 mg/kg/d. A total of 118 were treated for more than 6 months, and 114 more than a year. Similarly, from the published cases, there was 96 NKH patients out of 135 who were receiving a dose of 300-750 mg/kg/d. A total of 72 were treated for more than 6 months, and 61 more than a year.

The applicant has stated that "recurrent esophagitis and gastritis are apparently frequently observed AE". Furthermore, the applicant states this AE occurs if "benzoate preparation is not sufficiently diluted". There is no information presented as to the incidence of this AE, whether this AE is due to local irritation or what is the mechanism of action for the adverse event. This is of concern as there no assurance that the granule formulation currently presented will behave in a similar fashion to other oral formulations or indeed will be associated with an increased incidence of adverse events **LoQ**.

Serious and life-threatening adverse events were reported in patients receiving sodium benzoate including GI bleeding (2 cases), liver failure (10 cases+ 12 cases of liver cytolysis without liver failure), disseminated intravascular coagulation (5 cases), acute renal failure (3 cases), left ventricular dysfunction (1 case), cardiorespiratory arrest(1 case), seizures (36? cases), metabolic acidosis with coma (4 cases in case of overdose). A significant number of cases of liver failure, DIC and seizures is worrisome. As the analysis made by the applicant is based on the review of the literature cases it is difficult to establish relatedness of these adverse events to sodium benzoate. Further discussion in relation to information which needs to be included in section 4.8 of the SmPc is required **LoQ**.

As indicated by the applicant, hypokalaemia was one of the most frequently reported laboratory finding in the 25-year retrospective study of iv sodium benzoate in UCD patients. However, it is noted that hypokalemia is not listed in the SmPc. Other laboratory abnormalities reported include acidosis, hyponatremia. Sodium benzoate may cause hypernatremia due to its sodium content of sodium benzoate must be considered into account in the authorized dietary sodium intake.

It needs to be highlighted that benzoate treatment has a narrow, patient-dependent, therapeutic window, especially in subjects with NKH. If the amount of benzoate exceeds the availability of glycine, and glycine levels are below normal a significant toxicity may develop including development of metabolic acidosis with coma. The applicant recognizes this issue by adding the following recommendation to section 4.2 of the SmPC: "To prevent toxicity, the dose of benzoate should then be gradually increased, e.g. by 50 mg/kg/day, and plasma glycine and benzoate levels should be monitored before any subsequent dose change. The maximum daily dose should be less than 750 mg/kg/day." The applicant should further discuss this significant safety issue. The relevant justification for the proposed risk minimization measures and SmPc wording should be provided. In addition, it is considered that information provided in the SmPC is insufficient. The acceptable glycine and benzoate levels are not provided. Also, it should be specified how frequently glycine and benzoate levels should be checked, in what period after administration of sodium benzoate **LoQ**.

The requirement for validated assays of sodium benzoate measurement appears to be essential for the safe use of this drug. Especially in the neonatal period in UCD, where induction of hippurate synthesis may be delayed because of an immature N-acetylase and plasma benzoate concentrations may reach potentially toxic levels. Adequate assurance that these validated assays are available in the EU has not been provided **LoQ**.

The applicant has not provided any information on the potential differences in the safety profile of sodium benzoate in neonates as compared to older children and young adults. However, such differences are likely to occur taking into the consideration immaturity of enzymes responsible for the acylation of benzoate with CoA. As discussed above a significant variability in the metabolism of

sodium benzoate was observed in children and it cannot be concluded based in the provided data that the proposed doses of sodium benzoate are safe in this patient population **LoQ**.

5.5. Uncertainties and limitations about unfavourable effects

The applicant was asked to describe the methodology used to identify ADRs and justify the wording proposed for section 4.8of the SmPC. The applicant stated that all AEs identified in the literature were included in the proposed SmPC and no further justification of the proposed wording was provided, which is not acceptable The applicant included in the SmPC frequencies of the reported adverse reactions however the justification for these frequencies has not been provided. It is noted that some AEs reported in the literature are included in the SmPC and some AEs are not included and the reason for this is not clear **LoQ**.

The current presented information on safety is poor and precludes the making of a conclusion on clinical safety.

5.6. Effects Table

Due to the paucity of data it is not possible to generate an effects table.

5.6.1. Balance of benefits and risks

The benefit/risk balance is currently negative.

5.6.2. Additional considerations on the benefit-risk balance

- Not applicable.

5.7. Conclusions

The overall B/R of Prohippur is negative.