CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-562

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

CLINICAL PHARMACOLOGY REVIEW

NDA: 22-562	Submission Date: 18 JUN 2009
Submission Type; Code:	Original Submission; NME
Brand/Code Name:	Carbaglu
Generic Name:	Carglumic acid
Primary Reviewer:	Kristina Estes, Pharm.D.
Secondary Reviewer	Sue Chih Lee, Ph.D.
OCP Division:	Division of Clinical Pharmacology III
OND Division:	Division of Gastroenterology and Inborn Error of Metabolism
Sponsor:	Orphan Europe
Relevant IND(s):	61,265
Formulation; Strength(s):	200 mg tablet
Proposed Indication:	N-acetyl glutamate synthetase (NAGS) deficiency
Proposed Dosage Regimen:	Initial dose: 100-250 mg/kg/day in two to four divided doses

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1 Executive Summary

Carbaglu (containing carglumic acid or N-carbamyl-L-glutamic acid, NCGA) is intended for the treatment of hyperammonemia secondary to N-acetyl glutamate synthetase (NAGS) deficiency, a type of urea cycle disorder. Other agents, including Ammonul® and Buphenyl®, are approved for urea cycle disorders; however, the mechanism of action of Carbaglu is unique and may be of particular benefit to those patients with NAGS deficiency. The sponsor seeks to market the water-dispersible tablet in the U.S. and obtained approval from the EMEA in 2003 to market Carbaglu in Europe. NAGS deficiency is extremely rare (total number of patients worldwide is less than 50) and, before the availability of the proposed tablet formulation, patients were treated with NCGA in the form of a raw chemical or pharmaceutical grade product.

This NDA is submitted under the provisions of 505(b)(1). Due to the rarity of the disease, the sponsor did not perform standard Phase 3 studies but has provided a retrospective analysis of patients treated with Carbaglu as well as the associated case reports. To support the clinical pharmacology section of this NDA, the sponsor has submitted two Phase 1 studies, a bioequivalence study (OE 312/PK/99-01) to bridge the proposed tablet with the previously used pharmaceutical grade powder, and a mass balance study (b) (4) 313-1). The bioequivalence study is the source of much of the knowledge related to the pharmacokinetics of Carbaglu. However, due to methodological irregularities at the analytical site, results from the bioequivalence study are questionable. The sponsor has submitted some patient pharmacokinetic data consisting primarily of random plasma carglumic acid concentrations. Data from an in vitro study of drug metabolism conducted in human and rat hepatocytes have also been submitted.

The PDUFA due date of this NDA was extended for 3 months due to the submission of additional pharmacokinetic data from patients that, due to a formatting issue, was not able to be analyzed with the original submission.

DSI Inspection Report

An inspection of the analytical site showed variability in the matrix effect on quantitation of NCGA and 5-HPA when the analytes were extracted from matrix or mobile phase; therefore, the accuracy of the determination of the analytes in subject plasma could not be confirmed. The results from three experiments with failing precision and accuracy results were not reported and could have introduced bias in the results. In addition, the inter-run precision and accuracy at LLOQ was not accurately reported as not all method validation data were included in the statistical analysis. Other methodological irregularities were also identified in the DSI report, which is included in the Appendix (4.4). In summary, the pharmacokinetic data are questionable, which prevents the use of such information in the label.

1.1 Recommendations

From the viewpoint of the Office of Clinical Pharmacology, the Clinical Pharmacology and Biopharmaceutics information in the NDA is sufficient provided that mutual agreement on label language can be reached between the sponsor and

the Agency. Despite some deficiencies in the submission with regard to the characterization of carglumic acid pharmacokinetics, there is a reliable pharmacodynamic measurement with which to guide both dosing and response and sufficient clinical data was generated with the proposed tablets in patients.

1.2 Phase IV Commitments/Requirements

Due to the limited information regarding the metabolism of carglumic acid or the potential for drug interactions, we recommend an in vitro study to determine if carglumic acid is a substrate, inhibitor, or inducer of the cytochrome P450 enzyme system. This study should be conducted as a post-marketing requirement.

1.3 Summary of CPB Findings

Dose selection

The proposed starting dose of 100 to 250 mg/kg/day is based on clinical experience with the product. In practice, NAGS deficiency patients have received starting doses of 34 mg/kg/day up to 396 mg/kg/day in divided doses. The mean and median starting doses are 165.7 and 140 mg/kg/day, respectively, with an interquartile range of 106.65 to 205.5 mg/kg/day. The dose is then adjusted based on individual patient's response in plasma ammonia levels.

Dose-Response Relationship

Data from six patients in which dosing is available (range: 122 to 396 mg/kg/day) and for whom sufficient pre-dose ammonia levels (range: 85 to 242 μmol/L) and 24-hour post-dose ammonia levels (range 26 to 185 μmol/L) are documented does not demonstrate a clear dose-response relationship. Ammonia levels decline in all six patients by 24 hours; however, the magnitude of the decrease does not correlate with the dose administered. Furthermore, the rate of decline in plasma ammonia levels following the first dose is not well documented. The ability to establish a dose-response relationship is further confounded by the use of a protein-free diet in three of these patients while the other three were unrestricted in their protein intake.

As noted previously, the DSI inspection report identified methodological irregularities at the analytical site; therefore, the pharmacokinetic data generated from the bioequivalence study may not be reliable. A discussion of the results of all clinical pharmacology studies may still be found in the QBR portion of this review.

Note: Dose-proportionality, multiple-dose PK, food effect, TQT, and drug-drug interactions were not studied. These were discussed with the sponsor during the IND stage but were not requested in view of the circumstances surrounding the development of this drug (e.g., extremely low occurrence of the disease, dose titration for individual patients, and safety profiles of the drug). If situations arise in the future that may necessitate the collection of further information, we will reconsider the need of these studies.

2 QBR

The QBR was completed before the results of the DSI inspection were available. The whole QBR section is included for the purpose of documentation. As stated above, the DSI findings indicate the PK data obtained form the LC/MS/MS assay method presented in the QBR are questionable.

2.1 General Attributes of the Drug

2.1.1 What regulatory background or history information contributes to the assessment of the clinical pharmacology and biopharmaceutics of this drug?

Carbamylglutamic acid was designated as an orphan drug in January 1998 for the treatment of NAGS deficiency. In 2008, an original NDA for Carbaglu was submitted but subsequently withdrawn because the submission was not readily reviewable. Following the withdrawal of the original submission, the sponsor held several meetings with the Agency to address deficiencies that had been identified.

As part of the EMEA approval of Carbaglu in 2003, the sponsor committed to perform a mass-balance study in three volunteers to aid in the characterization of drug disposition in humans. The results of this mass-balance study as well as a bioequivalence study have been submitted with this NDA.

2.1.2 What are the highlights of the chemistry and physical-chemical properties of the drug substance, and the formulation of the drug product?

Carbaglu is formulated as a water-dispersible tablet. The components and composition are listed in the table below. The active ingredient, carglumic acid, is a chiral amino acid (L-isomer) with pKa values of 2.50, 3.55, and 8.60. The molecular weight is 190.06. Carglumic acid is stable in an alkaline medium (pH 10) but undergoes rapid degradation in very acidic medium (pH 1). Carglumic acid is slowly degraded when exposed to light or an oxidizing medium such as hydrogen peroxide. Principal degradation products are hydantoin-5-propionic acid (5-HPA) and diaza-1,3-dione-2,4-carboxy-7-cycloheptane.

Table 2. Composition of the proposed 200 mg carglumic acid tablet.

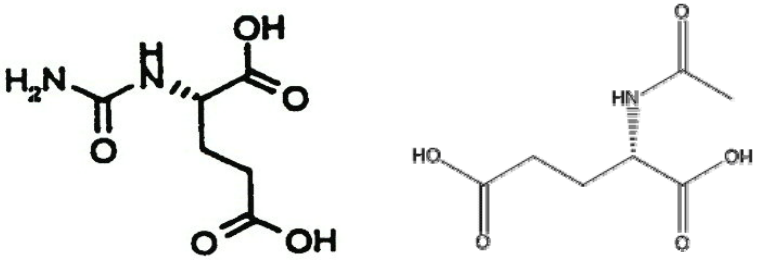
Names of ingredients	Unit formula	Function	Reference to standards
Active substance:			
Carglumic acid (N-carbamoyl-L-glutamic acid)	200.00 mg	active substance	In-house standards
Excipients:			
Cellulose, microcrystalline	(b) (4)	(b) (4)	USP, current Edition Ph. Eur. current Edition, 0316
Sodium lauryl sulfate			USP, current Edition Ph. Eur. current Edition, 0098
Hypromellose			USP, current Edition Ph. Eur. current Edition, 0348
Croscarmellose sodium			USP, current Edition Ph. Eur. current Edition, 0985
Silica, colloidal anhydrous			USP, current Edition Ph. Eur. current Edition, 0434
Sodium stearyl fumarate			USP, current Edition Ph. Eur. current Edition, 1567
(b) (4)			USP, current Edition Ph. Eur. current Edition, 0008
Total	500.00 mg	-	-

(b) (4)

2.1.3 What are the proposed mechanism of action and therapeutic indication(s)?

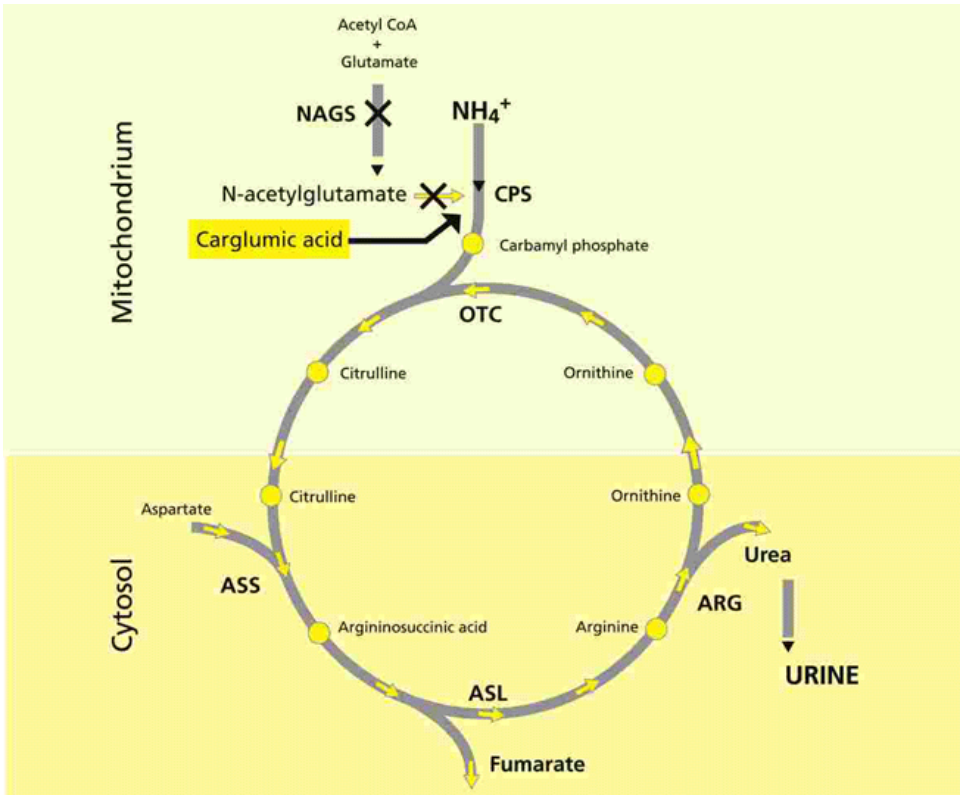
Carbaglu is a structural analogue of N-acetyl-L-glutamate, an obligatory cofactor of mitochondrial carbamoyl phosphate synthetase 1, the first enzyme of the urea cycle.

Figure 1. The chemical structure of N-carbamyl-L-glutamate (carglumic acid, on left) and N-acetyl-L-glutamate (on right).



In mammals, the urea cycle converts ammonia to urea in a five step process. The failure to activate this enzyme causes an accumulation of nitrogenous waste, mostly in the form of ammonia, causing hepatic encephalopathy.

Figure 2. The proposed mechanism of action of carglumic acid in NAGS deficiency patients.



From Orphan Europe [http://www.orphan-europe.com/Data/ModuleGestionDeContenu/03-Diseases/Hyperammonaemia/16.asp] accessed December 3, 2009.

Carglumic acid specifically addresses the underlying defect in NAGS patients but would be unlikely to be of any benefit in patients with other urea cycle disorders who have functional N-acetyl glutamate synthetase. Genetic testing may identify patients for whom this product would be beneficial.

2.1.4 What are the proposed dosage and route of administration?

The proposed starting dose is 100 to 250 mg/kg/day by mouth in divided doses. No specific maintenance dose has been proposed; however, in practice the maintenance doses are dictated by plasma ammonia levels and clinical response.

2.2 General Clinical Pharmacology

2.2.1 What are the design features of the pivotal clinical trials?

Due to the extreme rarity of the disease (less than 50 patients worldwide), the sponsor was not able to perform Phase 3 clinical trials of a randomized, double-blind, placebo-controlled nature. The sponsor has provided a retrospective review and case reports to support the use of Carbaglu in this orphan population.

2.2.2 What is the basis for selecting the response endpoints, i.e., clinical or surrogate endpoints, or biomarkers and how are they measured in clinical pharmacology and clinical studies?

NAGS deficiency results in elevated plasma ammonia levels which are closely correlated with the development of encephalopathy. Ammonia and other biomarkers, such as plasma citrulline, were monitored in patients and collected retrospectively. Due to the small number of patients, no Phase 3 clinical study was performed. Furthermore, ammonia levels were not studied in the clinical pharmacology studies as these were conducted in healthy volunteers. Plasma ammonia data is available in some patients prior to initiation of Carbaglu and at various time points after initiation of treatment; however, plasma ammonia was not consistently measured pre-dose or at specific intervals post-dose in all patients.

2.2.3 Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships? (if yes, refer to II. F, Analytical Section; if no, describe the reasons)

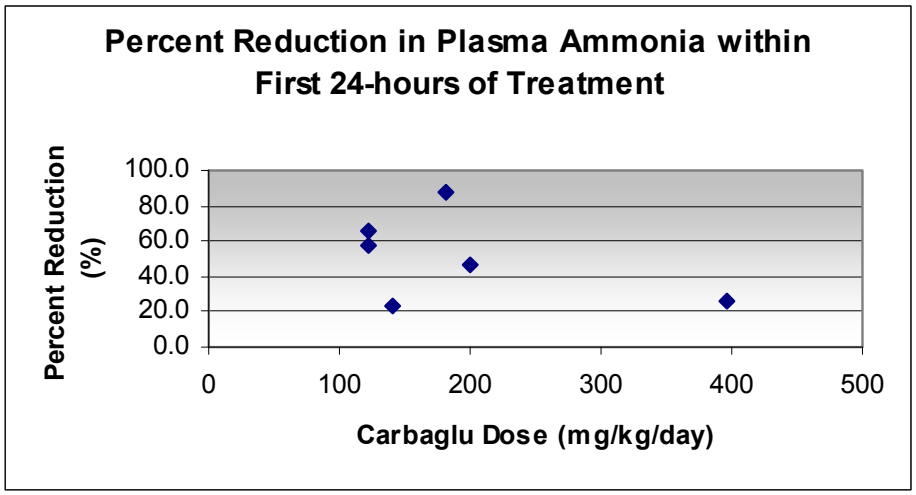
Carglumic acid can be assayed in both plasma and urine; however, no exposure-response relationship may be evaluated at this time due to the lack of available data. NAGS deficiency is a heterogeneous disease presenting with differing levels of severity and may be diagnosed anytime from the neonatal period (with a high degree of suspicion) into adulthood. Dose is therefore titrated to response based on plasma ammonia levels.

2.2.4 Exposure-response

2.2.4.1 What are the characteristics of the dose-response relationships for efficacy? If relevant, indicate the time to the onset and offset of the pharmacological response or clinical endpoint.

No dose-response relationship has been established for Carbaglu. Data from six patients in which dosing is available (range 122 to 396 mg/kg/day) and for whom sufficient pre-dose ammonia levels (range 85 to 242 μmol/L) and 24-hour post-dose ammonia levels (range 26 to 185 μmol/L) are documented does not show a clear dose-response relationship.

Figure 3. Dose-response in six patients following initial treatment with carglumic acid.



Ammonia levels decline in all six patients by 24 hours; however, the magnitude of the decrease does not correlate with the dose administered. Furthermore, the rate of decline in plasma ammonia levels following the first dose is not well characterized. The ability to establish a dose-response relationship is further confounded by the use of a protein-free diet in three of these patients while the other three were unrestricted in their protein intake.

The proposed starting dose of 100 to 250 mg/kg/day is based on clinical experience with the product. In practice, starting doses of 34 mg/kg/day up to 396 mg/kg/day in divided doses have been used in patients with suspected NAGS deficiency. If one includes all homozygous and heterozygous NAGS deficiency patients who received any form of carglumic acid, the mean and median starting doses are 165.7 and 140 mg/kg/day, respectively, with an interquartile range of 106.65 to 205.5. Maintenance dosing is based on plasma ammonia levels, not on plasma carglumic acid levels.

2.2.4.2 What are the characteristics of the exposure-response relationships for safety? If relevant, indicate the time to the onset and offset of the pharmacological response or clinical endpoint.

The relationship between exposure and long-term safety has not been established. The maintenance dosing for Carbaglu is generally lower than the starting dose; however, this data is not always clearly indicated in the patient records. Some NAGS deficiency patients have taken carglumic acid for many years without the occurrence of serious adverse events.

2.2.4.3 Does this drug prolong the QT or QTc interval?

Patients have not been shown to have prolonged QT interval as a result of Carbaglu treatment. A study of isolated canine Purkinje fibers showed no statistically significant effect on action potential parameters. Furthermore, in conscious dogs, no effect on QT interval or QTc was observed following administration of up to 1000 mg/kg carglumic acid. No thorough QT study in humans has been conducted. Given the lack of evidence of a cardiac effect following long-term use of Carbaglu in patients, we are not requesting the thorough QT study at this time.

2.2.4.4 Is the dose and dosing regimen consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?

The proposed starting dose of 100-250 mg/kg/day is based on clinical experience and there is little to no dose-response data upon which to base this recommendation. Plasma ammonia levels are easily measurable and provide a basis for titrating the Carbaglu dose following initial treatment. However, once the plasma ammonia levels are within the normal range, the rate of reduction in dose for maintenance therapy is not specified. Furthermore, in the event of an acute decompensation, there is no recommendation for adjusting the Carbaglu dose upwards.

The sponsor recommends that Carbaglu be administered in two to four divided doses. This recommendation is consistent with the observed half-life in healthy volunteers (6 hours in the initial elimination phase); however, the half-life in patients is not known.

2.2.5 What are the PK characteristics of Carbaglu?

The PK characteristics of Carbaglu are primarily derived from the bioequivalence study (OE312/PK/99-01) with some supporting data from the mass balance study (b) (4) 313-1). As noted previously, however, methodological irregularities at the analytical site of the bioequivalence study limit our certainty in these results.

Following administration of 100 mg/kg carglumic acid to 12 healthy volunteers, plasma concentrations of NCGA were measurable 0.5 hours post-dose and median t_{max} occurred at 3 hours (range: 1.5 to 4 hours) post-dose. Radioscintography also showed a peak, corresponding to the parent compound, at approximately 2-3 hours post-dose. The maximum plasma concentration for the tablet was 2708 ng/mL and the AUC was 22560 ng*h/mL and was not highly variable (CV: 31%).

The plasma concentration of NCGA shows a bi-exponential decline with 0-12 hours as the first phase and 12-24 hours as the second phase. The $t_{½}$ of the first phase is approximately 6 hours. The $t_{½}$ of the second phase could not be determined from the bioequivalence study due to the small number of blood samples available in the terminal phase. The specific metabolic pathway of NCGA has not been identified but results of the mass balance study suggest the ultimate end product may be CO_2. NCGA may also form a cyclization by-product, 5-HPA.

2.2.5.1 What were the results of the bioequivalence study?

A bioequivalence study in healthy male volunteers was performed to bridge the pharmacokinetic parameters of the pure chemical powder (administered to patients prior to the availability of the tablet) to the dispersible tablet formulation. Following administration of the tablet or the reference powder, plasma profiles of NCGA appear similar. The t_{lag} for each formulation is approximately 0.5 hours. The median t_{max} for the tablet formulation is 3 hours while the median t_{max} for the powder is 2 hours; however, the range for t_{max} is similar, occurring approximately 1.5 to 4 hours post-dose. The maximum plasma concentration for the tablet (2708 ng/mL) was similar to the powder (2943 ng/mL). The plasma concentrations of NCGA show a bi-exponential decline with an early phase elimination $t_{½}$ of approximately 6 hours. The 5-HPA plasma concentrations were all below or close to the limit of quantitation and urine concentrations were all below the limit of quantitation.

Table 3. Mean pharmacokinetic parameters of NCGA following administration of a single oral dose of 100 mg/kg.

N-Carbamyl-L-Glutamic acid	C_{max} (ng.ml)	t_{max} (h)	AUC_{0-t} (ng.ml.h)	$AUC_{0-\infty}$ (ng.ml.h)	$t_{1/2}$ (h)
Treatment A (Reference powder):					
Mean	2943		20850	22414	6.67
S.D.	839	1.5 - 4.0*	5297	5793	1.26
Median	2880	2.0	22085	23693	6.55
Treatment B (Dispersible tablet):					
Mean	2708		21126	22560	6.00#
S.D.	818	2.0 – 4.0*	6580	7019	1.50
Median	2550	3.0	19600	20559	5.56
Analysis of variance	NS (1)	NS (2)	NS (1)	NS (1)	NS (3)
90% confidence intervals	0.83-1.03		0.87-1.16	0.86-1.16	

*. Min and max value
#: N = 11 (b) (4)
1. Analysis of variance (PROC GLM) on log-transformed data
2. Wilcoxon signed rank test (PROC UNIVARIATE) on natural data
3. Analysis of variance (PROC GLM) on natural data

The point estimates for the C_{max} and AUC of the tablet to the reference powder are 0.92 and 1.01, respectively. Furthermore, the 90% confidence intervals for the point estimate of both parameters are within the acceptable range for bioequivalence. A review of the individual pharmacokinetic results shows the difference in AUC between the tablet and powder to be less than 25% in seven subjects. In the remaining five subjects for whom there is a greater than 25% difference in AUC between the two formulations, two subjects experienced a

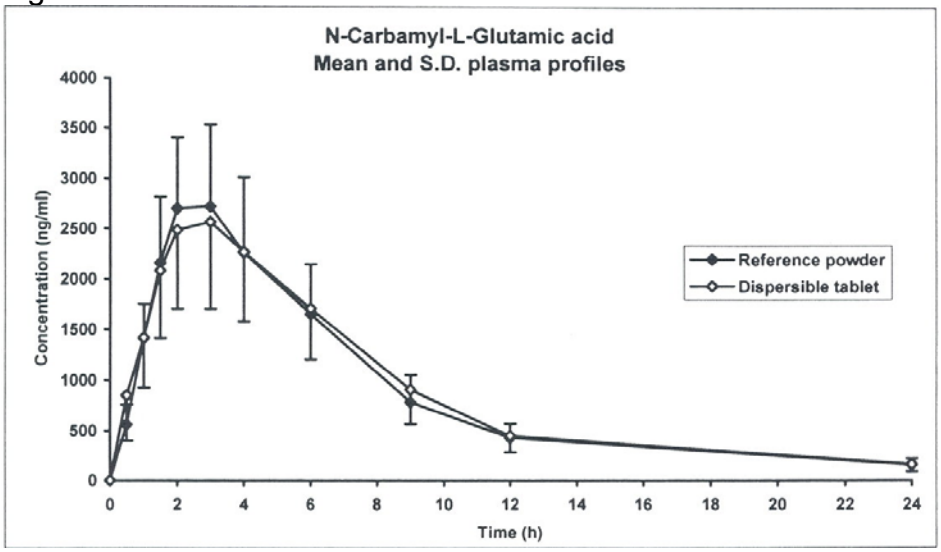
decrease and three subjects experienced an increase in exposure with the tablet relative to the powder.

Table 4. Additional pharmacokinetic parameters of NCGA following administration of a single oral dose of 100 mg/kg.

N-Carbamyl-L-Glutamic acid	**MRT** (h)	**Ae** (mg)	**Cltot/F** (ml/min)	**Vd/F** (L)	**Clr** (ml/min)
Treatment A (Reference powder):					
Mean	8.36	372	5784	3302	312
S.D.	1.09	82	1864	1114	91
Median	8.04	381	5010	3091	276
Treatment B (Dispersible tablet):					
Mean	8.04	360	5784	2783	295
S.D.	1.53	96	1742	1107	73
Median	7.82	330	5719	2657	290
Analysis of variance	NS (1)	NS (2)	NS (2)	NS (2)	NS (2)

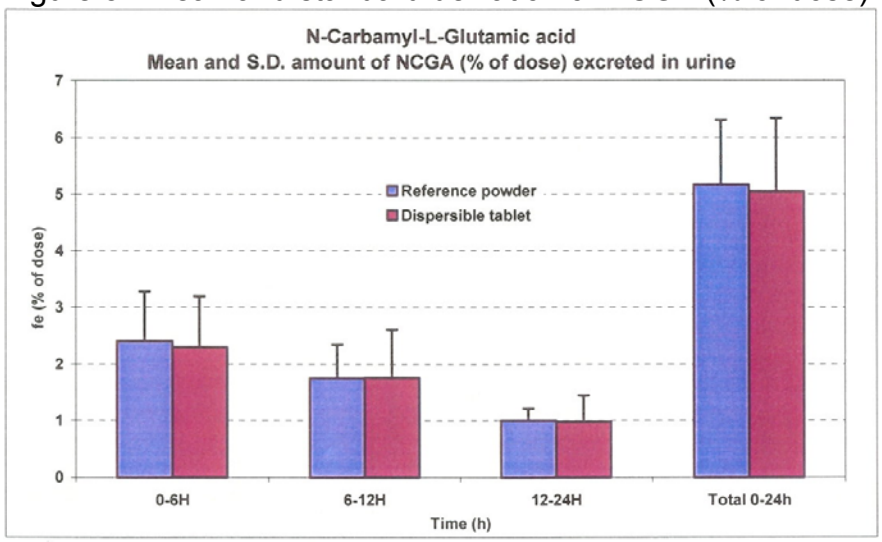
1. Analysis of variance (PROC GLM) on natural data
2. Analysis of variance (PROC GLM) on log-transformed data

Figure 4. Plasma concentration vs time for the test and reference formulations.



Following administration of the tablet or the reference powder, mean plasma profiles of NCGA appear similar. There is no apparent difference in t_{lag}, t_{max}, or C_{max} between the two formulations. As expected, there is also no difference in the elimination characteristics between the tablet and powder formulations.

Figure 5. Mean and standard deviation of NCGA (% of dose) excreted in the urine.

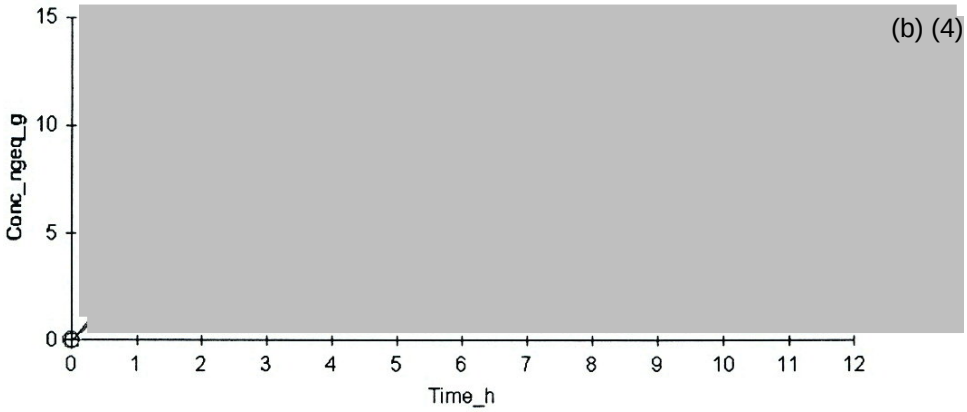


Approximately 5% of the dose is recovered in the urine as NCGA. There is no apparent difference in the percent of NCGA that is eliminated in the urine when administered as powder or tablet. In addition, there was also no significant difference in the amount of NCGA collected during the three periods during which urine was collected.

2.2.5.2 What were the results of the mass-balance study?

The mass balance study was conducted in three healthy male volunteers. Each subject received one radiolabeled oral dose of carglumic acid (100 mg/kg) following an overnight fast of at least 10 hours. Blood, urine, and feces were collected for 7 days post-dose. Breath CO_2 was also collected at selected time points over the first 24 hours.

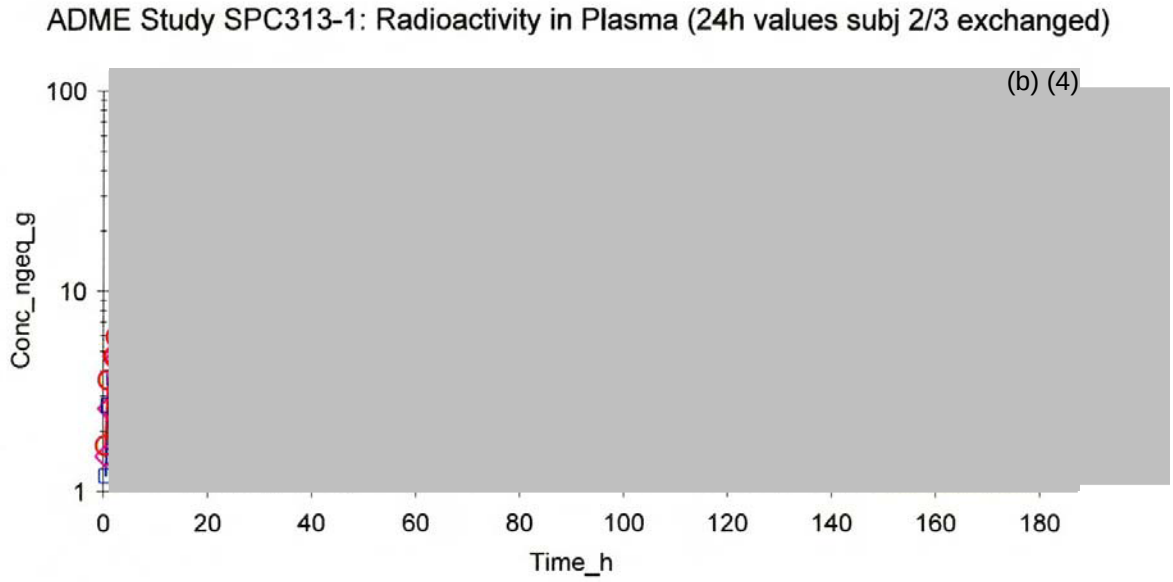
Figure 6. Plasma radioactivity profiles for 0-12 hours in three healthy volunteers following administration of a single oral dose of approximately 60 µCurie ^{14}C-labelled Carglumic acid.



The plasma concentration profile of radioactivity is characterized by a double peak. The first peak occurs approximately 2-3 hours post-dose while a second, higher peak occurs approximately 36-48 hours post-dose (see below). Upon further

analysis of the plasma samples, the concentration of parent compound peaked at 2.5 hours post-dose. Therefore, the higher radioactivity peak that is observed between 36 and 48 hours is not likely to be NCGA. The time at which the initial peak of radioactivity occurs is consistent with the t_{max} observed in the bioequivalence study.

Figure 7. Plasma radioactivity profiles for 0-168 hours in three healthy volunteers following administration of a single oral dose of approximately 60 µCurie ^{14}C-labelled Carglumic acid.



The second peak of radioactivity likely represents recycling of the amino acid carbon backbone, given the prolonged detection of plasma radioactivity and the low recovery of parent compound in these later samples. Enterohepatic recirculation of NCGA is possible but is less likely to adequately explain the appearance of the second peak.

Table 5. Percentage of radioactivity recovered in the urine, feces, and trapped CO_2, from three individuals following administration of a single oral dose of approximately 60 µCurie ^{14}C-labelled Carglumic acid.

Percent of Radioactivity Administered	Subject 1	Subject 2	Subject 3
Urine	8.68	8.41	9.75
Feces	71.96	16.45	72.67
Trapped volatiles	0.72	3.76	0.53
Total balance	***81.36***	***28.62***	***82.95***

The percent of radioactivity recovered in the urine is consistent among the three subjects and ranges from 8.41-9.75%. Further analysis identified only the parent compound in the urine samples. The percent of radioactivity recovered from feces is very similar in Subjects 1 & 3 (71.96% and 72.67%); however, only 16.45% of the total radioactivity was recovered from feces in Subject 2. The majority of the compound identified in feces was the NCGA but a small amount (approximately 1%) was identified as 5-HPA upon further analysis. Interestingly, the percentage of radioactivity recovered as trapped volatiles (as a percent of overall radioactivity administered) was significantly higher in Subject 2 relative to Subjects 1 & 3. The overall radioactivity recovered from Subject 2 was low (28.62% of the total dose administered) while plasma radioactivity remained significantly higher than the other two subjects. Although some radioactivity may have been lost, through missed fecal specimens for example, the data suggests that the radiolabeled carbons were recycled and incorporated into a biomaterial with a longer $t_{½}$ than NCGA.

2.2.5.3 How does the PK of the drug in healthy volunteers compare to that in patients?

The pharmacokinetics of carglumic acid were not fully assessed in NAGS deficiency patients. However, an analysis of six patients for whom sufficient pharmacokinetic data is available (total daily dose, dose per kg of body weight, dose per administration, plasma NCGA concentration, time of dose administration, and time of sample) who received a mean dose of 40 mg/kg (range: 7.4 to 122.3) resulted in a mean NCGA plasma concentration of 821 ng/mL (range: 282 to 2200) at the estimated t_{max} of 2 to 4 hours post-dose. This compares to a mean C_{max} of 2708 ng/mL in healthy volunteers who received 100 mg/kg as a single dose. The $t_{½}$ of carglumic acid in NAGS deficiency patients cannot be calculated with the available data. If the $t_{½}$ in patients is similar to that in healthy volunteers (6.6 hours), some accumulation may be anticipated with multiple daily doses.

A direct comparison of these data is difficult due to small number of blood samples available from patients, the wide range of doses administered, and the wide range of patient ages (neonate to 33 years of age).

2.2.5.4 What are the characteristics of drug distribution and clearance?

The apparent volume of distribution of carglumic acid is 2600-3000L and the total clearance is > 350 L/hr. Taken together, these data suggest the bioavailability of Carbaglu is low.

2.2.5.5 Does the mass balance study suggest renal or hepatic as the major route of elimination?

Approximately 5 to 10% of NCGA is excreted unchanged in the urine. Total fecal excretion of NCGA is approximately 72%, of which, 60% is excreted unchanged. Only 1% of dose is excreted in feces as 5-HPA, the cyclization by-product. The appearance of a second peak in plasma radioactivity in the mass balance study suggests there is significant recycling of the carbon backbone, although NCGA may also undergo enterohepatic recirculation. Ultimately, the carbon backbone of NCGA may be exhaled as CO_2.

Carglumic acid may not undergo metabolism by Phase 1 or Phase 2 enzymes in the liver based on the results of somewhat limited studies in rat and human liver hepatocytes. In this study, hepatocytes from two Sprague-Dawley rats and from two humans were incubated with aspartic acid and two concentrations of radiolabeled NCGA (25 μM and 250 μM, representing C_{max} and 10 times C_{max}, respectively) for up to 24 hours. LC/MS/MS and radioactive detection was performed to identify metabolites. The parent compound remained unchanged in all analyses. The activity of three enzymes, phenacetin deethylase (CYP1A2), nifedipine oxidase (CYP3A4), and paracetamol glucuronyltransferase (a Phase 2 enzyme), were measured as positive controls. Carglumic acid metabolism by other CYP enzymes may not be ruled out at this time.

2.2.5.6 Based on PK parameters, what is the degree of linearity or nonlinearity in the dose-concentration relationship?

The dose-proportionality of carglumic acid was not studied.

2.3 Intrinsic Factors

2.3.1 What intrinsic factors influence exposure and/or response and what is the impact of any differences in exposure on the pharmacodynamics?

The effect of various intrinsic factors on the pharmacodynamics of Carbaglu are not known. NAGS patients have been reported to require less Carbaglu on a mg/kg basis as their age increases; however, the impact of age on exposure has not been clearly demonstrated. Many polymorphisms have been identified in NAGS deficiency patients but the influence of a particular polymorphism on either exposure or response has not been established. Due to the small number of NAGS patients worldwide and the many polymorphisms associated with the disease, such a relationship is unlikely to be found and would not necessarily be useful in defining an initial or maintenance Carbaglu dose.

2.3.1.1 What is the status of pediatric studies and/or any pediatric plan for study?

NAGS deficiency is often diagnosed in neonates (with a high index of suspicion) or in infancy. As such, Carbaglu has been administered to pediatric patients from as early as the first few days of life.

2.3.1.2 What pregnancy and lactation use information is there in the application?

There are no adequate and well controlled studies in pregnant women. Studies in rats and rabbits demonstrated no teratogenic, embryotoxic, or fetotoxic effects when carglumic acid was administered in doses of 500 to 2000 mg/kg/day. Carglumic acid is present in the milk of lactating rats.

2.4 Extrinsic Factors

2.4.1 What extrinsic factors (drugs, herbal products, diet, smoking, and alcohol use) influence exposure and/or response and what is the impact of any differences in exposure on pharmacodynamics?

The influence of smoking, alcohol use, and other extrinsic factors on the exposure or PD of Carbaglu is not known. NAGS deficiency patients may utilize a protein restricted diet in the overall management of hyperammonemia; however, the effect of such a diet on the exposure or PD of Carbaglu is not known.

2.4.2 Drug-Drug Interactions

2.4.2.1 Is there an in vitro basis to suspect in vivo drug-drug interactions?

There is no apparent in vitro basis to suspect a drug-drug interaction via Phase 1 or Phase 2 enzymes; however, the in vitro studies of the cytochrome P450 enzyme system were limited and did not address the potential for P450 inhibition or induction. Carglumic acid was not metabolized in studies utilizing human and rat hepatocytes. Studies to assess the affinity of NCGA for P-glycoprotein or other transport proteins were not conducted.

2.4.2.2 Does the label specify co-administration of another drug and, if so, has the interaction potential between these drugs been evaluated?

The label does not specify co-administration of Carbaglu and another drug. In clinical practice, however, Carbaglu is likely to be used in conjunction with other ammonia lowering agents in the treatment of acute hyperammonemia. No drug-drug interaction studies have been conducted but in vitro studies suggest a low potential for interactions via Phase 1 or Phase 2 enzymes.

2.4.2.3 What other co-medications are likely to be administered to the target patient population?

Current treatments available for NAGS deficiency are the same as those available for other urea cycle disorders. These agents that increase nitrogen excretion include sodium benzoate, sodium phenylacetate, and sodium phenybutyrate. Concomitant use of these agents is most likely to occur in the event of acute hyperammonemia.

2.4.2.4 Is there a known mechanistic basis for pharmacodynamic drug-drug interactions?

The primary pharmacodynamic endpoint is plasma ammonia level. Administration of agents that may cause hyperammonemia include valproate, carbamazepine, phenobarbital, and topiramate. Notably, these are all antiepileptic agents and seizures are reported to be common in patients with urea cycle disorders. Other potential pharmacodynamic interactions, via inhibition of N-acetyl glutamate synthetase or another urea cycle enzyme for example, have not been identified.

2.4.3 What issues related to dose, dosing regimens or administration are unresolved?

The sponsor recommends dividing the total daily dose into two to four doses; however, this recommendation is not clearly based on the PK or PD parameters of Carbaglu as plasma ammonia and NCGA were not measured at consistent times relative to dose administration in patients. It is possible that this recommendation is based upon tolerability (some patients require large numbers of tablets per day) rather than an issue of efficacy. The sponsor also recommends Carbaglu be administered before meals or feeding but the effect of food on NCGA exposure is not known. Lastly, the directions for administering Carbaglu to patients are not clear. Carbaglu was administered as a suspension in the Phase 1 studies and the label indicates the tablet may be dispersed in water; however, it is unclear if patients may be advised to swallow the tablet whole as an alternative to the suspension.

2.5 General Biopharmaceutics

2.5.1 What is the in vivo relationship of the proposed to-be-marketed formulation to the pivotal clinical trial formulation in terms of comparative exposure?

No pivotal clinical trials were conducted. NAGS deficiency patients have been treated with pure chemical and pharmaceutical grade carglumic acid prior to the availability of the dispersible tablet formulation. A BE study in healthy male volunteers was performed to bridge the pharmacokinetic parameters of the pharmaceutical grade powder to the dispersible tablet formulation. Following administration of the tablet or the reference powder, plasma profiles of NCGA appear similar and the 90% CI of the AUC and C_{max} are within the acceptable range for bioequivalence.

The tablet formulation used in the bioequivalence study contained (b) (4) sodium lauryl sulfate (SLS) per tablet but the sponsor subsequently (b) (4) the SLS content to (b) (4) secondary to concerns about the incidence of GI complaints in the group who received the investigational product. Based upon the results of dissolution studies, the difference is SLS content is not likely to significantly alter the PK of the to-be-marketed formulation relative to the product utilized in the bioequivalence study.

2.5.2 What is the effect of food on the bioavailability (BA) of the drug from the dosage form? What dosing recommendation should be made, if any, regarding administration of the product in relation to meals or meal types?

The sponsor also recommends Carbaglu be administered before meals or feeding but the effect of food on NCGA exposure is not known.

2.6 Analytical Section

2.6.1 How are the active moieties identified and measured in the plasma in the clinical pharmacology and biopharmaceutics studies?

Plasma and urine NCGA and hydantoin-5-propionic acid (5HPA), a possible impurity, were quantified by LC/MS/MS. Tetradeuterated NCGA and

tetradeuterated hydantion-5-propionic acid were used as internal standards. No specific metabolites have been identified; therefore, the PK of only the parent compound and the possible impurity were characterized.

2.6.1.1 What is the range of the standard curve? How does it relate to the requirements for clinical studies? What curve fitting techniques are used?

The range of the standard curve for NCGA in plasma was 50 to 10,000 ng/mL. The range of the standard curve for H5PA in plasma was 10 to 2000 ng/mL. The range of the standard curve for both compounds in urine was 20 to 1000 μg/mL. Only two of the three QC concentrations were representative of the study samples for NCGA in the healthy volunteers. Few subjects had measurable H5PA, which may reflect a low level of exposure to the impurity or the inability of the analytical methods to accurately characterize H5PA below 20 ng/mL due to high levels of background noise. The calibration curves were linear with a mean coefficient of correlation r of 0.9998 for both compounds.

2.6.1.2 What are the lower and upper limits of quantification (LLOQ/ ULOQ)?

The lower limit of quantitation is 50 ng/mL for NCGA in plasma, 10 ng/mL for hydantion-5-propionic acid in plasma, and 20 mcg/mL for both compounds in urine. The upper limit of quantitation is 10,000 ng/mL for NCGA in plasma, 2000 ng/mL for hydantion-5-propionic acid in plasma, and 1000 mcg/mL for both compounds in urine.

2.6.1.3 What is the accuracy, precision and selectivity at these limits?

Extraction recovery in plasma was > 87% for both NCGA and H5PA. For NCGA, the inter-run precision was characterized by a CV ≤ 6.91% and mean accuracy was within 90.7 and 103% of the theoretical values. For H5PA, the inter-run precision was characterized by a CV ≤ 11.5% and mean accuracy was within 91.9 and 98% of the theoretical values.

An inspection of the analytical site showed variability in the matrix effect on quantitation of NCGA and H5PA when the analytes were extracted from matrix or mobile phase; therefore, the accuracy of the determination of the analytes in subject plasma could not be confirmed. The results from three experiments with failing precision and accuracy results were not reported and could have introduced bias in the results. In addition, the inter-run precision and accuracy at LLOQ was not accurately reported as not all method validation data were included in the statistical analysis.

2.6.1.4 Under what conditions were the samples collected and what is the sample stability under the conditions used in the study?

Blood samples were collected into heparinized tubes. Samples were centrifuges at 4°C at 1100g for 10 minutes immediately after collection. The plasma was transferred and stored at -80°C until analysis. Urine was collected in 10mL aliquots and frozen until analysis. Stability for both NCGA and hydantion-5-propionic acid

in plasma after three freeze/thaw cycles and two concentration levels. The plasma samples were stable over a run of approximately 6.2 hours.

3 Detailed Labeling Recommendations

(b) (4)

4 Appendices

5 Page(s) of Draft Labeling has been Withheld in Full immediately following this page as B4 (CCI/TS)

4.2 Individual Study Reviews

NDA 22562
Carbaglu (Carglumic acid, 200mg tablets)
Orphan Europe SARL
Submission Date: June 18, 2009

Study Title: ***Comparative Pharmacokinetic Study of OE312 in Healthy Male Volunteers After Single Oral Administration*** (OE312/PK/99-01).

Background
N-acetyl glutamate synthetase (NAGS) deficiency is a very rare hereditary disorder of the urea cycle, in which ammonia fails to be converted to urea resulting in neurotoxic hyperammonemia. N-carbamyl-L-glutamic acid [NCGA, aka carbamylglutamate (CG)] is an analogue of N-acetyl glutamate (NAG), the activator of the first enzyme in the urea cycle, carbamyl phosphate synthetase (CPS). Although CG has a lower affinity for CPS, the mitochondrial membrane is more permeable to CG than NAG and CG is less prone to cytosolic aminoacylase than NAG.

Since 1980, a very small number of patients have been treated with NCGA, either in the form of a raw chemical or a pharmaceutical grade product. Orphan Europe has developed a dispersible tablet that they plan to market. The comparative bioavailability study was necessary to bridge this new tablet formulation with the powdered product that was used in the clinical trials.

Study Objectives
The primary objective of the study was to determine the relative bioavailability and tolerance of NCGA from two OE312 formulations, the new dispersible tablet versus the reference powder, administered as a single oral dose of 100 mg/kg in 12 healthy volunteers. Secondary objectives include determining the PK parameters of NCGA in plasma and urine.

Study Design
This was a randomized, single-dose, open-label, two-way crossover study to compare two formulations of NCGA in 12 healthy male volunteers. Following a 2-week run-in phase, two single 100 mg/kg doses were administered with a 7-day washout period in between doses. Doses were administered following a 10-hour fast. The first meal was not served until four hours post-dose.

Dose and Formulation
The dose selected for this study was 100 mg/kg, which is in the range of starting doses for Carbaglu. The formulation used in this Phase 1 study contained (b) (4) sodium lauryl sulfate per tablet; however, the sponsor subsequently (b) (4) the SLS content to (b) (4) secondary to concerns about the incidence of GI complaints in the group who received the investigational product. Both the tablet and reference powder were administered as a suspension in 250 mL of water.

Inclusion Criteria
Participants had to be Caucasian males between the ages of 18 and 30 years. The weight of each subject had to be within 10% of their ideal body weight. Laboratory measurements and ECG had to be within the normal range. In addition, subjects had to be non-smokers (or smoke lass than 10 cigarettes per day) and have normal eating habits.

Exclusion Criteria
Subjects who had a history of major medical or psychiatric illness were excluded from participation. Those who regularly used sedatives, tranquilizers, hypnotics, alcohol, or who drank excessive quantities of coffee, tea, or other caffeinated beverages were also excluded. Use of any OTC product within one month of the study or use of a treatment that could affect hepatic microsomal enzymes was also cause for exclusion.

Demographics

Twelve healthy male volunteers met all the inclusion and exclusion criteria and completed the study. The mean age of participants was 23.3 ± 3.3 years (range: 18 to 28 years). The mean body weight was 72.1 ± 3.1 kg (range: 65.9 to 75 kg).

Pharmacokinetic Monitoring
Blood samples were drawn prior to dosing and at 0.4, 1, 1.5, 2, 3, 4, 6, 9, 12, and 24 hours post-dose. Urine was collected at baseline and at the following intervals: 0-6, 6-12, and 12-24 hours post-dose. Pharmacodynamics and pharmacogenetics were not studies.

Pharmacokinetic Results
Following administration of the powder, plasma concentrations of NCGA were measurable 0.5 hours post-dose and t_{max} occurred approximately 1.5 to 4 hours post-dose. The maximum plasma concentration for the tablet (2708 ng/mL) was similar to the powder (2943 ng/mL). The plasma concentrations of NCGA show a bi-exponential decline with 0-12 hours as the first phase and 12-24 hours as the second phase. The linear trapezoidal method was used to calculate the AUC_t.

Mean pharmacokinetic parameters of NCGA following administration of a single oral dose of 100 mg/kg.

N-Carbamyl-L-Glutamic acid	**C_{max}** (ng.ml)	**t_{max}** (h)	**AUC_{0-t}** (ng.ml.h)	**$AUC_{0-\infty}$** (ng.ml.h)	**$t_{1/2}$** (h)
Treatment A (Reference powder):					
Mean	2943		20850	22414	6.67
S.D.	839	1.5 - 4.0*	5297	5793	1.26
Median	2880	2.0	22085	23693	6.55
Treatment B (Dispersible tablet):					
Mean	2708		21126	22560	6.00#
S.D.	818	2.0 – 4.0*	6580	7019	1.50
Median	2550	3.0	19600	20559	5.56
Analysis of variance	NS (1)	NS (2)	NS (1)	NS (1)	NS (3)
90% confidence intervals	0.83-1.03		0.87-1.16	0.86-1.16	

*. Min and max value
: N = 11 (b) (4)
1. Analysis of variance (PROC GLM) on log-transformed data
2. Wilcoxon signed rank test (PROC UNIVARIATE) on natural data
3. Analysis of variance (PROC GLM) on natural data

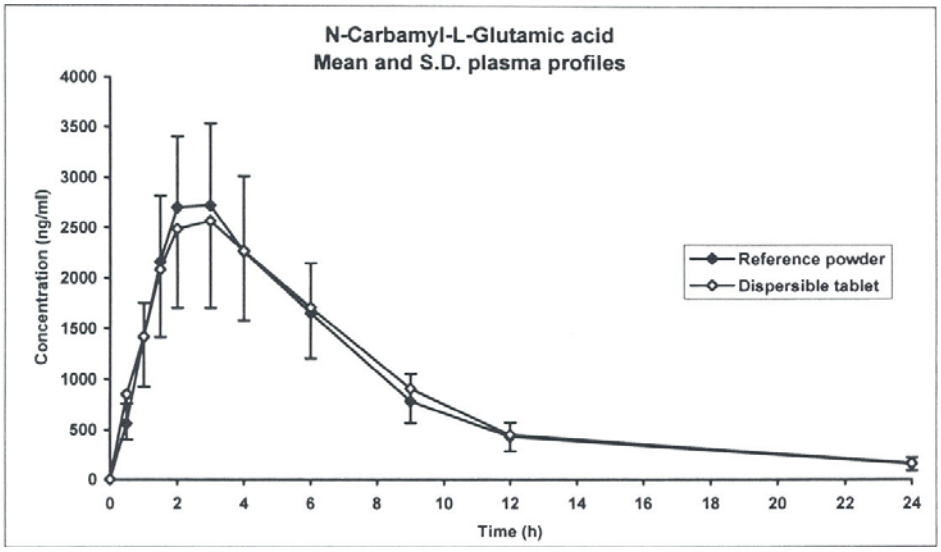
The point estimates for the C_{max} and AUC of the tablet to the reference powder are 0.92 and 1.01, respectively. Furthermore, the 90% confidence intervals for the point estimate of both parameters are within the acceptable range for bioequivalence. The t_{max} was also similar between the two treatment groups. A review of the individual plasma profiles shows virtually no difference between the tablet and powder products in seven subjects. A decrease in exposure with the tablet relative to the powder was seen in three subjects, while an increase in exposure was seen in two subjects with the tablet relative to the powder.

Additional pharmacokinetic parameters of NCGA following administration of a single oral dose of 100 mg/kg.

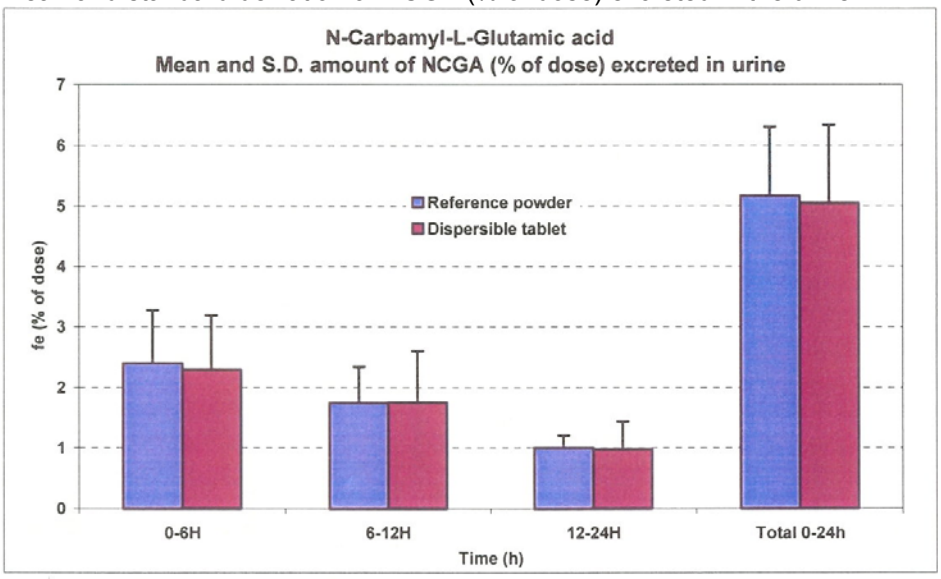
N-Carbamyl-L-Glutamic acid	MRT (h)	Ae (mg)	CItot/F (ml/min)	Vd/F (L)	Clr (ml/min)
Treatment A (Reference powder):					
Mean	8.36	372	5784	3302	312
S.D.	1.09	82	1864	1114	91
Median	8.04	381	5010	3091	276
Treatment B (Dispersible tablet):					
Mean	8.04	360	5784	2783	295
S.D.	1.53	96	1742	1107	73
Median	7.82	330	5719	2657	290
Analysis of variance	NS (1)	NS (2)	NS (2)	NS (2)	NS (2)

1. Analysis of variance (PROC GLM) on natural data
2. Analysis of variance (PROC GLM) on log-transformed data

Plasma concentration vs time for the test and reference formulations.



Mean and standard deviation of NCGA (% of dose) excreted in the urine.



Bioanalytical Assay

Plasma and urine NCGA and hydantoin-5-propionic acid were quantified by LC/MS/MS. The limit of quantitation was 50 ng/mL for N-Carbamyl-L-Glutamic acid in plasma, 10 ng/mL for hydantion-5-

propionic acid in plasma, and 20 mcg/mL for both compounds in urine. Tetradeuterated N-Carbamyl-L-Glutamic acid and tetradeuterated hydantion-5-propionic acid were used as internal standards. For N-Carbamyl-L-Glutamic acid QC samples, the mean accuracy was between 90.7% and 103% and the inter-day precision CV was less than 6.91%. For hydantion-5-propionic acid QC samples, the mean accuracy was between 91.9 and 98.0%, and the inter-day precision CV was less than 11.5%.

Statistical Plan
All quantitative variables were summarized with descriptive statistics. C_{max} and AUC were analyzed by ANOVA on the logarithmically transformed data. The 90% CI limits for relative treatment differences were calculated by geometric means based on the logarithmic transformation of the intraindividual ratios of Cmax and AUC and had to fall within the bioequivalence range for AUC and a wider range (0.70-1.43) for C_{max}.

Safety
Overall, 10 adverse events were observed during the study of which 9 were probably related to treatment. These included 8 episodes of liquid stools and one episode of dyspepsia. Three subjects experienced an AE with the reference powder and five subjects experienced an AE while taking the tablet.

Sponsor's Conclusion
The tolerability of the new tablet formulation was comparable to the reference powder. The mean C_{max} of NCGA was not significantly different between formulations. The 90% confidence interval of the point estimate for C_{max} (0.83 to 1.03) is within the range for bioequivalence. Mean AUC_t and AUC_∞ were not significantly different between formulations. The 90% CI for the point estimate for AUC_∞ (0.86 to 1.16) is within the range for bioequivalence. The range of t_{max} was similar for both formulations. Plasma concentrations of NCGA declined in a bi-exponential manner with the first phase occurring from 0-12 hours and the second phase occurring after 12 hours. The mean $t_{½}$ of approximately 6 hours is likely to underestimate the true $t_{½}$.

Reviewer's Comments

- *The rate and extent of absorption of the tablet is similar to that of the reference powder formulation. A review of the individual plasma profiles did not reveal any concerning performance issues related to the tablet. The median t_{max} was slightly longer for the tablet; however, the ranges for each formulation were similar.*

NDA 22562
Carbaglu (Carglumic acid, 200mg tablets)
Orphan Europe SARL
Submission Date: June 18, 2009

Study Title: ***An open label study with ^{14}C-labelled carglumic acid to investigate the mass balance, pharmacokinetics and metabolism following a single oral administration to healthy male subjects*** (b) (4) **313-1)**.

Background
Carbaglu was approved by the EMEA in 2003. As part of that approval, the sponsor committed to perform a mass-balance study in three volunteers to aid in the characterization of drug disposition in humans. Prior to the conduct of the mass balance study, the best characterization of NCGA pharmacokinetics was from the bioequivalence study which had been performed to bridge the proposed tablet formulation to the pure chemical powder that had been used by patients before the tablet became available.

Study Objectives
The primary objective of the study was to evaluate the mass balance, pharmacokinetics, and metabolism of carglumic acid given as single oral dose of 60 µCurie ^{14}C-labeled carglumic acid (100 mg/kg) in 3 healthy male volunteers. The secondary objective was to evaluate the tolerability of the formulation of ^{14}C-labelled carglumic acid.

Study Design
This was a single-center, single-dose, open-label, mass-balance study in 3 healthy male volunteers. Each subject received one radiolabeled oral dose of carglumic acid (100 mg/kg) following an overnight fast of at least 10 hours. Blood, urine, and feces were collected for 7 days post-dose. Breath CO_2 was also collected at selected time points over the first 24 hours.

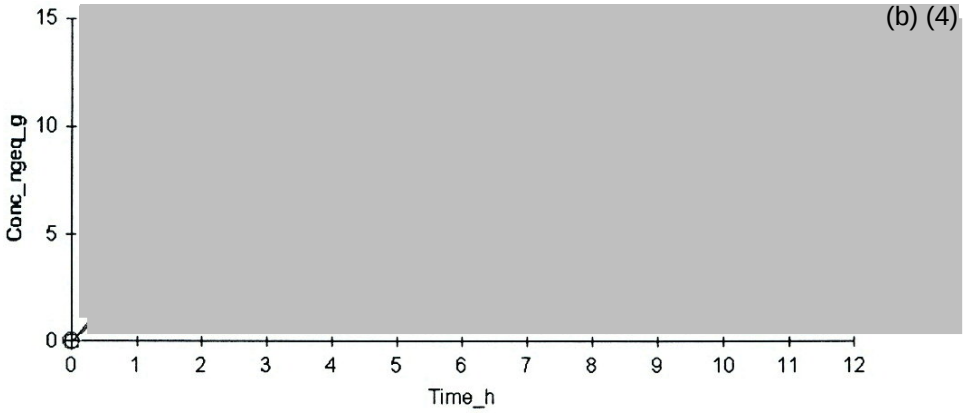
Inclusion Criteria
Participants had to be males between the ages of 40 and 55 years. The weight of each subject was to be between 50 and 95 kg and had to be within -10% to +20% of their ideal body weight. Volunteers had to have acceptable blood pressure (systolic 110-160 mmHg, diastolic BP 65-95 mmHG) and pulse rate (50-100 bpm).

Exclusion Criteria
Subjects who had a history of major medical or psychiatric illness and those who had a history of serious adverse reactions or hypersensitivity to any drug were excluded from participation. History of alcohol or drug abuse within the last 5 years was also cause for exclusion. Subjects who smoked more than 10 cigarettes per day were also excluded.

Demographics
Three healthy male Caucasian volunteers met all the inclusion and exclusion criteria and completed the study. The mean age of participants was 46.7 years (range: 46-47 years). The mean body weight was 81.2 kg (range: 77.2-87.7 kg).

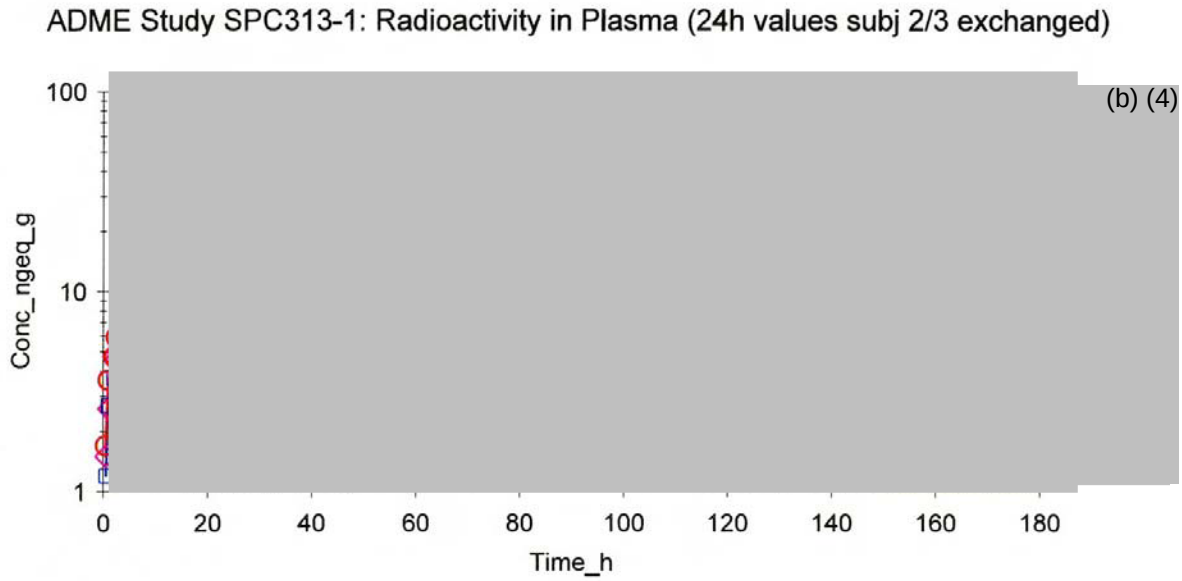
Pharmacokinetic Monitoring
Blood samples were drawn prior to dosing and at 1, 2, 4, 6, 8, 10, 12, 16, 24, 36, 48, 72, 96, 120, 144, & 168 hours post-dose. Urine was collected at baseline and at the following intervals: 0-2, 2-4, 4-8, 8-12, 12-24, 24-36, 36-48 hours post-dose and once daily thereafter up to Day 7. Feces was collected as passed.

Pharmacokinetic Results
Plasma radioactivity profiles for 0-12 hours in three healthy volunteers following administration of a single oral dose of approximately 60 µCurie ^{14}C-labelled Carglumic acid.



The plasma concentration profile of radioactivity was characterized by a double peak. The first peak occurred approximately 2-3 hours post-dose while a second, higher peak occurred approximately 36-48 hours post-dose (see below).

Plasma radioactivity profiles for 0-168 hours in three healthy volunteers following administration of a single oral dose of approximately 60 µCurie ^{14}C-labelled Carglumic acid.



Mean pharmacokinetic parameters of radioactivity following administration of a single oral dose of approximately 60 µCurie ^{14}C-labelled Carglumic acid.

Parameter for plasma concentrations (radioactivity expressed in µg-eq/g)	(N = 3)	(N = 3) 24h value subject 002/003 exchanged
AUC (0-inf) [h·µg-eq/g]	4250 ± 3617 (3032) [1399 - 8318]	4277 ± 3900 (2717) [1399 - 8715]
%AUC [%]	38.39 ± 6.26 (36.36) [33.39 – 45.41]	39.13 ± 5.59 (37.26) [34.70 – 45.41]
AUC (0 - tn) [h·µg-eq/g]	2692 ± 2339 (2020) [764 - 5293]	2720 ± 2616 (1705) [764 - 5691]
C_{max} [µg-eq/g]	37.53 ± 29.06 (41.00) [6.90 – 64.70]	30.40 ± 30.38 (19.60) [6.90 – 64.70]
$t_{1/2}$ [h]	107.54 ± 23.17 (100.31) [88.84 – 133.46]	107.54 ± 23.17 (100.31) [88.84 – 133.46]
t_{max} [h]	32.0 ± 6.93 (36.00) [24.0 – 36.0]	40.0 ± 6.93 (36.00) [36.0 – 48.0]

The percent of radioactivity recovered in the urine was consistent among the three subjects and ranged from 8.41-9.75%. The percent of radioactivity recovered from feces was very similar in two subjects (71.96% and 72.67%); however, only 16.45% of the total radioactivity was recovered from feces in the third subject.

Analysis of Radioactivity
Radioactivity was determined on Packard liquid scintillation counters. The pre-dose sample of each subject was measured as a control of the background radiation. The method for characterizing radioactivity in whole blood, plasma, urine, and feces was reliable with all QC samples between 99.4 and 100.2% of expected.

Statistical Plan
All quantitative variables were summarized with descriptive statistics. .

Safety
No serious adverse events were reported. One subject experienced two mild adverse events, loose stools and one episode of tiredness, that were regarded as possibly related to the study drug.

Sponsor's Conclusion
The mechanism of elimination of the radioactivity from the central compartment seems to be similar in all three subjects. Radioactivity from urine ranged from 8.41 to 9.75% of the total radioactivity administered. The terminal half-lives of the decline in radioactivity in both plasma and urine are fairly uniform in all three subjects. The first peak of radioactivity (2.5 to 3 hours) is consistent with the t_{max} observed in the bioequivalence study. The sponsor also concludes that erythrocytes do not take up or bind much radioactivity.

Regarding the peculiarities of the results for Subject 2, the sponsor suggests this subject had much higher absorption of radioactivity from the GI tract secondary to long stay of the chyme in the terminal colon. Subject two produced the least amount of feces and had the lowest frequency of feces production. The sponsor also suggests that Subject 2 may have excreted the major portion of radioactivity in exhaled air, which was only measured for the first 24 hours. Overall, the sponsor concludes that the period for feces and urine sampling may not have been long enough and about half of the radioactivity in blood was excreted from the body after the subjects had left the unit.

Reviewer's Comments
The t_{max} for radioactivity is consistent with the t_{max} observed in the bioequivalence study. Urinary excretion of radioactivity was consistent between subjects. Fecal excretion was similar for two of the three subjects while the other subject appeared to have much lower fecal excretion of radioactivity during the 168 hour observation period. This subject had a significantly higher excretion of radioactivity in the form of exhaled CO2 relative to the other two subjects. As CO2 was

only collected for the first 24 hours, it is possible that a significant amount of radioactivity lost by to this route of elimination in the 24-168 hour period. It is also possible that collecting urine and feces for a longer period of time may have aided in characterizing the fate of NCGA in this volunteer.

4.3 Cover Sheet and OCPB Filing/Review Form

Office of Clinical Pharmacology

New Drug Application Filing and Review Form

General Information about the Submission

	Information		Information
NDA/BLA Number	**22-562**	**Brand Name**	**Carbaglu**
OCP Division (I, II, III, IV, V)	**III**	**Generic Name**	**Carglumic Acid**
Medical Division	**GI**	**Drug Class**	
OCP Reviewer	**Kris Estes**	**Indication(s)**	**Hyperammonemia due to NAGS deficiency**
OCP Team Leader	**Sue Chih Lee**	**Dosage Form**	**Tablet**
Pharmacometrics Reviewer		**Dosing Regimen**	**Initial Dose: 100-250 mg/kg/day in 2-4 divided doses**
Date of Submission	**18 JUN 2009**	**Route of Administration**	**PO**
Estimated Due Date of OCP Review		**Sponsor**	**Orphan Europe**
Medical Division Due Date		**Priority Classification**	
PDUFA Due Date	**18 MAR 2010**		

Clin. Pharm. and Biopharm. Information

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	**X**			
Tabular Listing of All Human Studies	**X**			
HPK Summary	**X**			
Labeling	**X**			
Reference Bioanalytical and Analytical Methods	**X**			
I. Clinical Pharmacology				
Mass balance:	**X**	**1**		
Isozyme characterization:				
Blood/plasma ratio:				
Plasma protein binding:				
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:	**X**	**1**		
multiple dose:				
Patients-				
single dose:				
multiple dose:				
Dose proportionality -				
fasting / non-fasting single dose:				
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:				
In-vitro:				
Subpopulation studies -				
ethnicity:				
gender:				

pediatrics:				
geriatrics:				
renal impairment:				
hepatic impairment:				
PD -				
Phase 2:				
Phase 3:				
PK/PD -				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:	**X**	**1**		**Retrospective Case Series**
Population Analyses -				
Data rich:				
Data sparse:				
II. Biopharmaceutics				
Absolute bioavailability				
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:				
replicate design; single / multi dose:	**X**	**1**		
Food-drug interaction studies				
Bio-waiver request based on BCS				
BCS class				
Dissolution study to evaluate alcohol induced dose-dumping				
III. Other CPB Studies				
Genotype/phenotype studies				
Chronopharmacokinetics				
Pediatric development plan				
Literature References				
Total Number of Studies		**4**		

On **initial** review of the NDA/BLA application for filing:

	Content Parameter	Yes	No	N/A	Comment
Criteria for Refusal to File (RTF)					
1	Has the applicant submitted bioequivalence data comparing to-be-marketed product(s) and those used in the pivotal clinical trials?	X			
2	Has the applicant provided metabolism and drug-drug interaction information?	X			
3	Has the sponsor submitted bioavailability data satisfying the CFR requirements?	X			
4	Did the sponsor submit data to allow the evaluation of the validity of the analytical assay?	X			
5	Has a rationale for dose selection been submitted?	X			Rationale provided for initial dose but not for maintenance dose.
6	Is the clinical pharmacology and biopharmaceutics section of the NDA organized, indexed and paginated in a manner to allow substantive review to begin?	X			
7	Is the clinical pharmacology and biopharmaceutics section of the NDA legible so that a substantive review can begin?	X			
8	Is the electronic submission searchable, does it have			X	

	appropriate hyperlinks and do the hyperlinks work?				
Criteria for Assessing Quality of an NDA (Preliminary Assessment of Quality)					
	Data				
9	Are the data sets, as requested during pre-submission discussions, submitted in the appropriate format (e.g., CDISC)?		X		Column headings need clarification from sponsor.
10	If applicable, are the pharmacogenomic data sets submitted in the appropriate format?			X	
	Studies and Analyses				
11	Is the appropriate pharmacokinetic information submitted?	X			
12	Has the applicant made an appropriate attempt to determine reasonable dose individualization strategies for this product (i.e., appropriately designed and analyzed dose-ranging or pivotal studies)?	X			
13	Are the appropriate exposure-response (for desired and undesired effects) analyses conducted and submitted as described in the Exposure-Response guidance?		X		
14	Is there an adequate attempt by the applicant to use exposure-response relationships in order to assess the need for dose adjustments for intrinsic/extrinsic factors that might affect the pharmacokinetic or pharmacodynamics?			X	Dose adjustment made purely on plasma ammonia levels.
15	Are the pediatric exclusivity studies adequately designed to demonstrate effectiveness, if the drug is indeed effective?	X			
16	Did the applicant submit all the pediatric exclusivity data, as described in the WR?			X	
17	Is there adequate information on the pharmacokinetics and exposure-response in the clinical pharmacology section of the label?		X		
	General				
18	Are the clinical pharmacology and biopharmaceutics studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?	X			
19	Was the translation (of study reports or other study information) from another language needed and provided in this submission?	X			

IS THE CLINICAL PHARMACOLOGY SECTION OF THE APPLICATION FILEABLE?
Yes.

If the NDA/BLA is not fileable from the clinical pharmacology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Kristina Estes	27 DEC 2009
Reviewing Clinical Pharmacologist	Date
Team Leader/Supervisor	Date

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Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22562	ORIG-1	ORPHAN EUROPE	CARBAGLU (CARGLUMIC ACID)

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

KRISTINA E ESTES
02/22/2010
Revised 2/22/10

SUE CHIH H LEE
02/24/2010

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

Office of Clinical Pharmacology

New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA/BLA Number	**22-562**	**Brand Name**	**Carbaglu**
OCP Division (I, II, III, IV, V)	**III**	**Generic Name**	N-Carbamyl-L-Glutamic acid
Medical Division	**DGP**	**Drug Class**	
OCP Reviewer	**Insook Kim, Ph.D.**	**Indication(s)**	For the treatment of NAGS deficiency
OCP Team Leader	**Sue-Chih Lee, Ph.D.**	**Dosage Form**	**Dispersible Tablet 200 mg**
Pharmacometrics Reviewer		**Dosing Regimen**	100-250 mg/kg/day in divided doses
Date of Submission	**6/18/2009**	**Route of Administration**	**Oral**
Estimated Due Date of OCP Review		**Sponsor**	Orphan Europe SARL
Medical Division Due Date		**Priority Classification**	**P**
PDUFA Due Date	**12/18/2009**		

Clin. Pharm. and Biopharm. Information

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	**X**			
Tabular Listing of All Human Studies	**X**			
HPK Summary	**X**			
Labeling	**X**			
Reference Bioanalytical and Analytical Methods	**X**	**2**		
I. Clinical Pharmacology				
Mass balance:	**X**	**1**		**N=3**
Isozyme characterization:				
Blood/plasma ratio:				
Plasma protein binding:				
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:				
multiple dose:				
Patients-				
single dose:				
multiple dose:	**X**	**1**		**Random PK sampling**
Dose proportionality -				
fasting / non-fasting single dose:				
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:				
In-vitro:				
Subpopulation studies -				
ethnicity:				
gender:				
pediatrics:				
geriatrics:				

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renal impairment:				
hepatic impairment:				
PD -				
Phase 2:				
Phase 3:				
PK/PD -				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:				
Population Analyses -				
Data rich:				
Data sparse:				
II. Biopharmaceutics				
Absolute bioavailability				
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:	**X**	**1**		**OE312/PK/99-01**
replicate design; single / multi dose:				
Food-drug interaction studies				
Bio-waiver request based on BCS				
BCS class				
Dissolution study to evaluate alcohol induced dose-dumping				
III. Other CPB Studies				
Genotype/phenotype studies				
Chronopharmacokinetics				
Pediatric development plan				
Literature References				
Total Number of Studies		**4**		

On **initial** review of the NDA/BLA application for filing:

	Content Parameter	Yes	No	N/A	Comment
Criteria for Refusal to File (RTF)					
1	Has the applicant submitted bioequivalence data comparing to-be-marketed product(s) and those used in the pivotal clinical trials?	X			
2	Has the applicant provided metabolism and drug-drug interaction information?			X	
3	Has the sponsor submitted bioavailability data satisfying the CFR requirements?	X			
4	Did the sponsor submit data to allow the evaluation of the validity of the analytical assay?	X			
5	Has a rationale for dose selection been submitted?	X			
6	Is the clinical pharmacology and biopharmaceutics section of the NDA organized, indexed and paginated in a manner to allow substantive review to begin?	X			
7	Is the clinical pharmacology and biopharmaceutics section of the NDA legible so that a substantive review can begin?	X			
8	Is the electronic submission searchable, does it have appropriate hyperlinks and do the hyperlinks work?			X	
Criteria for Assessing Quality of an NDA (Preliminary Assessment of Quality)					

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

	Data				
9	Are the data sets, as requested during pre-submission discussions, submitted in the appropriate format (e.g., CDISC)?			X	
10	If applicable, are the pharmacogenomic data sets submitted in the appropriate format?			X	
	Studies and Analyses				
11	Is the appropriate pharmacokinetic information submitted?	X			
12	Has the applicant made an appropriate attempt to determine reasonable dose individualization strategies for this product (i.e., appropriately designed and analyzed dose-ranging or pivotal studies)?		X		
13	Are the appropriate exposure-response (for desired and undesired effects) analyses conducted and submitted as described in the Exposure-Response guidance?		X		
14	Is there an adequate attempt by the applicant to use exposure-response relationships in order to assess the need for dose adjustments for intrinsic/extrinsic factors that might affect the pharmacokinetic or pharmacodynamics?		X		
15	Are the pediatric exclusivity studies adequately designed to demonstrate effectiveness, if the drug is indeed effective?			X	
16	Did the applicant submit all the pediatric exclusivity data, as described in the WR?			X	
17	Is there adequate information on the pharmacokinetics and exposure-response in the clinical pharmacology section of the label?			X	
	General				
18	Are the clinical pharmacology and biopharmaceutics studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?	X			
19	Was the translation (of study reports or other study information) from another language needed and provided in this submission?	X			

IS THE CLINICAL PHARMACOLOGY SECTION OF THE APPLICATION FILEABLE?
_____Yes__

If the NDA/BLA is not fileable from the clinical pharmacology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Insook Kim, Ph.D.	7/21/2009
Reviewing Clinical Pharmacologist	Date
Sue-Chih Lee, Ph.D.	7/21/2009
Team Leader/Supervisor	Date

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

Filing Memo

Submitted is an original NDA for Carbaglu pursuing the indication of the specific treatment of hyperammonemia due to the deficiency of the hepatic enzyme N-acetylglutamate synthase (NAGS deficiency). In support the sponsor submitted two full study reports of clinical pharmacokinetics i.e. a mass balance study and a comparative bioavailability study and random assessment of systemic exposure in patients during treatment. The clinical efficacy and safety is supported by a collection of separate patient treatment record without controlled clinical trials due to rarity of patients. The proposed dosage regimen is to initiate at 100-250 mg/kg/day in divided doses within a day and adjust the dose based on plasma ammonia levels.

Recommendation:

We recommend that this submission be filed. Although there are many deficiencies in this submission, the applicant submitted pharmacokinetic information of the formulation used in clinical trial and bridging information between the clinical formulation and the to-be-marketed formulation. Therefore, we think that the applicant met the minimum requirement and other deficiencies should be review issues.

Information requests (item 1-5) to be conveyed to the sponsor

- 1) Please, provide SAS file for individual PK parameters for healthy subjects. If such information is already submitted please guide the review to the location. The format and definition of each variable should be separately provided and clearly explained. For example, in your previous submission of SAS file of PK in patients dated April 30, 2008. The format of date of 1st dose-day 1 (code: ddday11) was defined as DDMMYY. However, the actual data in SAS file was 16056 for a subject PATNO16 as such this information was not interpretable.

  2) Please, provide information of dose administered to patients in the tables for plasma concentrations in patients and plasma ammonia and glutamate levels at baseline and during/after treatment (pages 19 and 20 in section 2.7.)

  3) Please, provide new copy of below information as current copies are illegible.
  Module 2: Section 2.7.Table "Results of the assays" in page 22 of 53
  Section 2.6 Figure 11
  Module 5: Report 8, Table 4 in volume 1.5.

  4) It is noted that genotyping was conducted for patients during treatment. Please, provide relevant information including but not limited to genotyping methods, genotyping results and effect of genotype on the responses to treatment such as plasma ammonia and glutamate levels change. If such information is already submitted, please guide the reviewer to the location of the information.

  5) Please, clarify how the powder formulation was administered to patients in relation to meal e.g. before or after meals?

Main studies to be reviewed are as follows:

- Comparative Bioavailability in Healthy Volunteers
  A clinical trial in healthy male volunteers was conducted to compare the bioavailabi1ity of the formulated dispersible tablet (Carbaglu) and the pure powder of NCGA. One single dose at the

usual therapeutic level was chosen. This trial also exposed the elimination plasma kinetics and urine excretion of the unchanged compound. Additionally, the level of a potential degradation resulting impurity and/or metabolite was assessed.

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- Mass balance Study in Healthy Volunteers
  Another clinical trial using ^{14}C radio-labeled carg1umic acid was performed at the same dose in thee male healthy volunteers in order to determine the mass balance and possible metabolism of carglumic acid.

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- CGA plasma concentrations in Treated Patients
  Three laboratory determinations were performed in treated patients. Thee different reports were generated.

Clinical Pharmacology and Biopharmaceutics program has many deficiencies identified upon preliminary review as follows:

- Multiple dose PK
- Dose proportionality assessment
- Food effect study
- Assessment of drug interaction potential and metabolism pathway

Potential review issues upon preliminary review are as follows:

- How the to-be-marketed formulation comparable to clinical formulation?
  - For treatment of patients, pure powder was administered to patients. On the other hand, the to-be-marketed formulation is a dispersible tablet which would be dispersed in (b) (4) mL water prior to ingestion. The sponsor conducted a relative bioavailability study between powder and a dispersible tablet; however, the to-be-marketed dispersible tablet is further modified to (b) (4), sodium lauryl sulfate from (b) (4) per tablet. According to a CMC reviewer Dr. Marie Kowblansky, this change in formulation would normally require an in vivo bioavailability study for bridging. However, because the formulation would be taken dispersed in water; no in vivo bioavailability is warranted. On the surface, the comparative dissolution between powder and the to-be-marketed formulation is comparable. The effect of SLS on oral absorption appears to be minimal as the systemic exposure between powder and the formulation with (b) (4) SLS apparently was bioequivalent. As such the effect of (b) (4) SLS content in the to-be-marketed formulation on oral bioavailability is expected to be insignificant. However, because neither the to-be-marketed formulation nor the (b) (4) SLS formulation was not used for patients treatment, the safety of daily consumption of (b) (4) SLS on a long-term basis (250 mg/kg/day for 70 kg BW) should be addressed.

- Is the proposed dose adequate?
  - The proposed dosage regimen is to initiate at 100-250 mg/kg/day in divided doses within a day and adjust the dose based on plasma ammonia levels.

- Mass balance study was conducted only in three subjects so the reliability of the results is a review issue.

- Is the proposed dosage administration supported?
  - The current proposal is to divide the total daily dose into two to four doses to be given before meals or feeding and that the tablets may be dispersed in a minimum of (b) (4)

of water and ingested immediately or administered through a syringe via a nasogastric tube.

- What the effect of genotype on the treatment effect?

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

Clinical ADME			
Comparative bioavailability and pharmacokinetic study in healthy male volunteers after single oral administration of unlabelled carglumic acid (study ref. OE 312/PK/99-01)	**Report 1**	Final study report Aster/Cephac ref. P99148 dated April 2001 : Comparative bioavailability study of OE 312 in healthy male volunteers after single oral administration	Module 5
	Report 2	Bioanalytical report (b) (4) ref. 026/00 029 dated March 2001 and amended in September 2001: Assays of N-Carbamoyl-L-Glutamic acid in human plasma and urine collected during a kinetic trial. Products *N-Carbamoyl-L-Glutamic acid and hydantoin-5-propionic acid determination in plasma and in urine*	Module 5
The evidence of NCGA metabolites in human and rat hepatocytes was studied. N-Carbamyl 14C-L-Glutamic acid was synthesised. Cultures were then conducted with hepatocytes obtained from two rats (Sprague-Dawley rats) and from two human donors.	**Report 3**	Evidence of N-Carbamyl-L-Glutamic acid metabolites in human and rat hepatocytes. (b) (4) study report No 026/00 080 - Volume 1, May 2001. Volume 2, March 2001	Module 5
Mass balance, pharmacokinetic and metabolism study in healthy male volunteers after single oral administration of ^{14}C-labelled carglumic acid (Study ref. [redacted]313-1) and interspecies ADME report	**Report 4**	An open label study with ^{14}C-labelled carglumic acid to investigate the mass balance, pharmacokinetics and metabolism following a single oral administration to healthy male subjects [redacted] Final study report ref. (b)313-1 - April 2004 Radioactivity measurements report RCC ref. 84096 dated December 2003 and amended in February 2004 and in March 2004: [^{14}C] carglumic acid (Carbaglu): Determination of total amounts of radioactivity in blood, plasma, urine and feces of healthy male subjects.	Module 5

	Report 5	Bioanalytical report (b) (4) 026/02 070 dated November 2004: Metabolism study of carglumic acid after a single oral administration of [^{14}C] carglumic acid to three human volunteers.	Module 5
	Report 6	Additional bioanalytical report (b) (4) 026/04 022 dated September 2004: Validation and assays of carglumic acid in human urine using low calibration curve.	Module 5
	Report 7	Additional study report (b) (4) 026/04 023 dated September 2004 (Original study report in French): (Supplementary research on the metabolism of carglumic acid after oral administration of a single dose of ^{14}C-carglumic acid to three healthy volunteers).	
	Report 8	DATA REVIEW – Absorption, distribution, metabolism and elimination of Carglumic acid in animals and humans- Cross species metabolic profile comparison [redacted] April 2009	Module 5
Pharmacokinetic data in patients			
Assays in plasma and urine samples of 2 patients	**Report 9**	Bioanalytical report (b) (4) ref. 026/01 028 dated September 2001: Assays of N-Carbamyl-L-Glutamic acid in plasma and urine samples collected in patients under treatment. Test items: *N-Carbamyl-L-Glutamic acid and hydantoin-5-propionic acid*	Module 5
Assays in plasma samples of patients	**Report 10**	(b) (4) report No. 026/01 070 dated February 2009 Final version n°2: Assays of N-Carbamyl-L-Glutamic acid in plasma and urine samples collected in patients under treatment.	Module 5
	Report 11	(b) (4) report No. 026/06 110 dated February 2009 Final version n°2: Carglumic acid and 5 HPA assays in human plasma specimens collected during a patients' follow-up program - Determination of the presence of diaza in the specimens	Module 5

Retrospective, non-comparative, descriptive review of data collected from 23 NAGS deficiency patients treated with carglumic acid on a long term basis. Evaluation of neurological and psychomotor status, and anthropometric development (growth) parameters; the analysis of the implementation of restrictive /free protein diet and concomitant treatment; analysis of the carglumic doses prescribed as an indirect efficacy parameter and as a definition of the dose response determination; brief pharmacokinetic analysis and a description of safety data in those NAGS deficiency patients	**Report 12**	Carbaglu Retrospective Data Review in NAGS Deficiency Patients. Part I: Clinical and Biological Responses of Patients with NAGS Deficiency to Acute and Chronic Treatment with Carglumic Acid. Report and Appendices A to K (appendix F is a CD) Part II: Individual Patient Narratives in NAGS Deficiency Patients Orphan Europe Report written by Dr Carlos Camozzi (April 2009)	Module 5
Determination of restoration of ureagenesis in 2 NAGS deficiency, 4 PA (propionic acidemia) and 1 asymptomatic NAGS heterozygote patients during 3-day treatment with Carglumic Acid (Carbaglu) as evidenced by $^{13}C/^{15}N$ incorporation into urea, concentrations of plasma ammonia, urea and amino acids.	**Report 13**	Experience with carglumic acid (Carbaglu) in the US - Ureagenesis restoration in hyperammonemic patients - Mendel Tuchman *et al.*	Module 5
Safety report on all patients receiving carglumic acid, regardless the underlying disease, for acute and chronic treatment	**Report 14**	Clinical safety report on carglumic acid covering the period of 1 January 1991 to 31 December 2008. Orphan Europe Report (16 March 2009)	Module 5
QT report in patients and HV	**Report 15**	Overall data review report for the risk of QT prolongation, covering the period from 1 January 1991 to 31 March 2009 Orphan Europe report (20 April 2009)	Module 5

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/s/

Insook Kim
7/23/2009 10:18:05 AM
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Sue Chih Lee
7/23/2009 01:44:58 PM
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