CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-562

PHARMACOLOGY REVIEW(S)

Comments on N 22-562 Carbaglu carglumic acid
From Abby Jacobs, AD
Date: 3/15/10

1. I concur that there are no pharm/tox issues with approval and the labeling of pharm/tox portions is acceptable, with the changes suggested by the reviewer.

2. I do not concur with the postmarketing requests
a. There is no need for a chronic study in nonrodents in addition to the rodent chronic study

-The number of animals per group will be small
-There are human data sufficient for approval.
-The animals tested will not have the condition of hyperammonemia, and thus adverse effects observed at high doses may not be relevant to the patient population
-the drug is lifesaving
- the number of persons having this condition is rather small

b. There is no need for a carcinogenicity study
The animals tested will not have the condition of hyperammonemia, and thus adverse effects observed at high doses may not be relevant to the patient population
-the drug is lifesaving and
- the number of persons having this condition is rather small

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

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/s/

ABIGAIL ABBY C C JACOBS
03/15/2010

MEMORANDUM **DEPARTMENT OF HEALTH AND HUMAN SERVICES**
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

FROM: David B. Joseph, Acting Pharmacology Team Leader

DATE: March 11, 2010

SUBJECT: NDA 22,562 (serial # 000 dated June 17, 2009)

Sponsor: Orphan Europe, SARL

Drug Product: Carbaglu (carglumic acid)

Comments:

1. Carbaglu (carglumic acid, or N-carbamoyl-L-glutamic acid) was developed for the treatment of hyperammonemia due to N-acetylglutamate synthase (NAGS) deficiency. Carglumic acid is a structural analog of N-acetyl-L-glutamate (NAG), which is an obligatory allosteric activator of mitochondrial carbamoyl phosphate synthetase 1 (CPS 1), the first enzyme of the urea cycle. NAG is synthesized by the enzyme NAGS. In the absence of NAGS, NAG is not produced and plasma levels of ammonia are elevated due to impaired function of the urea cycle. NAGS deficiency is the rarest of the hereditary urea cycle disorders. The time of onset of clinical signs in NAGS deficiency patients is variable, ranging from shortly after birth through adulthood. The neonatal onset of NAGS deficiency is severe, with death expected to occur within a few days. Carglumic acid acts as a replacement for NAG in NAGS deficiency patients by activating CPS 1.

2. The nonclinical dataset did not identify any safety issues that would impact the approvability of carglumic acid. The most notable findings in the nonclinical data were the high mortality in orally-treated neonatal rats, and the impaired growth and survival of rat pups in a peri-/post-natal developmental study. In a 2-week oral toxicity study, administration of 2000 mg/kg/day produced deaths in most rat pups (neonates) within 2-3 days, whereas drug-related deaths were not observed at lower dose levels (250-1000 mg/kg/day). The lethal dose in this study is 8 times the maximum recommended starting dose for Carbaglu (250 mg/kg), based on a bodyweight comparison (mg/kg).

   Since the cause of hyperammonemia in neonates is usually unknown and because of the length of time needed to obtain a diagnosis, it is expected that carglumic acid will be administered in neonates with hyperammonemia as an adjunctive treatment with other ammonia lowering therapies (e.g., sodium phenylbutyrate). In this scenario, treatment with carglumic acid would likely continue until a cause of hyperammonemia is identified (e.g., a specific urea cycle enzyme deficiency). Therefore, the observed toxicity in orally-treated neonatal rats and in the offspring of carglumic acid-treated rats is a safety concern.

However, this concern can be addressed in the labeling (see the Pharmacology/Toxicology review by Dr. Yuk-Chow Ng).

3. General toxicology studies were conducted in rats only, although the Agency did request submission of a chronic toxicity study in a nonrodent species. The Sponsor has not provided any convincing rationale for the omission of toxicity studies in a nonrodent species. This deficiency is discussed in detail in Dr. Ng's review. I concur with Dr. Ng's recommendation that the Sponsor should conduct a chronic (9-month) oral toxicity study in a nonrodent species, as a post-marketing requirement.

4. I concur with Dr. Ng's recommendation that the Sponsor should conduct a 2-year carcinogenicity study in a single species, as a post-marketing requirement. The Sponsor has committed to performing this study following the approval of Carbaglu.

Recommendations:

There are no nonclinical issues which preclude the approval of Carbaglu for treatment of hyperammonemia due to NAGS deficiency. I concur with Dr. Ng's recommendation for approval, and with the recommendations for post-marketing nonclinical studies, as described above.

_____ _____

David B. Joseph, Ph.D. Date
Acting Pharmacology Team Leader
Division of Gastroenterology Products

cc:
NDA 22,562
DGP
DGP/CSO
DGP/Dr. Joseph
DGP/Dr. Ng
OND IO/Dr. Jacobs

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

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/s/

DAVID B JOSEPH
03/11/2010

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-562**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**6/17/2009**
PRODUCT:	**Carbaglu®**
INTENDED CLINICAL POPULATION:	**Patients with hyperammonemia associated with N-Acetylglutamate synthase deficiency**
SPONSOR:	**Orphan Europe** **Paris, France**
DOCUMENTS REVIEWED:	**Vol. 1-15**
REVIEW DIVISION:	**Division of Gastroenterology Products**
PHARM/TOX REVIEWER:	**Yuk-Chow Ng, Ph.D.**
ACTING PHARM/TOX TEAM LEADER:	**David B. Joseph, Ph.D.**
DIVISION DIRECTOR:	**Donna Griebel, M.D.**
PROJECT MANAGER:	**Roland Girardet, MHS, MS, MBA**

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

The application is recommended for approval.

B. Recommendation for nonclinical studies

The Applicant should conduct a chronic (9-month) oral toxicity study in a nonrodent species and a 2-year carcinogenicity study in a single species, as post-marketing requirements.

C. Recommendations on labeling

Recommendations are shown below for the following sections and subsections: "Pregnancy", "Nursing Mothers", "OVERDOSAGE", "Pharmacodynamics", "Carcinogenesis, Mutagenesis, Impairment of Fertility", and "Animal Toxicology and/or Pharmacology".

Established Pharmacologic Class

The Sponsor did not propose an established pharmacologic class.

Sponsor's Proposed Version:

(b)(4)

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

(b) (4)

(b) (4)

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

In safety pharmacology studies, no significant effects on blood pressure, heart rate, or ECG were noted in conscious dogs after single oral doses of up to 1000 mg/kg carglumic acid. The drug had no significant effects on cardiac action potential in isolated canine purkinje fibers at concentrations of up to 0.1 mM.

Carglumic acid also had no significant effects on CNS or respiratory functions in rats at oral doses up to 1000 mg/kg.

In rats, dogs, and humans, most of the drug remains unchanged after oral dosing, with a small fraction (up to 9%) metabolized and excreted as CO_2. The excretion of carglumic acid in rats and dogs differs from that observed in humans. In humans, a greater percentage of carglumic acid was eliminated in feces (approximately 70%) compared to rats and dogs (18-35%). Two distinct plasma radioactivity peaks were observed in humans after oral administration of [^{14}C]carglumic acid, whereas only one peak was observed in animals. The significance of this difference between humans and animals is uncertain. In human studies, [^{14}C]L-glutamic acid and [^{14}C]hydantoin-5-propionic acid, in addition to [^{14}C]carglumic acid, were detected in the feces. Glutamic acid is considered as a potential metabolite of carglumic acid, and hydantoin-5-propionic acid is both a known impurity (i.e. degradation product) in carglumic acid and a potential metabolite. Studies in rats and dogs failed to detect any metabolite of carglumic acid, aside from CO_2.

In acute toxicity studies, single oral doses of up to 2800 mg/kg carglumic acid were generally well tolerated in rats. Similarly, in acute intravenous studies in rats, doses of up to 238 mg/kg were well tolerated.

2-Week and 26-week oral toxicity studies were conducted in rats. The 2-week study was conducted in neonatal rats, using dose levels of 0 (vehicle), 250, 500, 1000, and 2000 mg/kg/day on days 4 to 21 postpartum. All doses of carglumic acid were associated with mortality. However, the deaths at the lower doses (250-1000 mg/kg/day) were considered to be the result of gavage errors. The high dose produced clinical signs in animals prior to death (e.g., coldness to touch, pallor of body extremities, emaciation, dehydration, swollen abdomen, and hypokinesia). Most animals in the 2000 mg/kg/day group died after 2-3 days of treatment, and the deaths in this group are considered as drug-related. Carglumic acid produced a decrease in bodyweight gains at 1000 and 2000 mg/kg/day, while physical and reflex development was unaffected at up to 1000 mg/kg/day. Carglumic acid also produced reductions in BUN at 1000 mg/kg/day, and in urine pH at 250 mg/kg/day (6.5, as compared to 7.5 in control group). At 1000 mg/kg/day, the only significant histopathologic finding was the dilated kidney pelvis in male rats.

In the 26-week oral toxicity study, carglumic acid was administrated to juvenile rats at 0 (vehicle), 500, and 1000 mg/kg/day. At 500 and 1000 mg/kg/day, interstitial mononuclear cell aggregation in kidney was observed. The high dose produced necrotizing inflammation in Harderian gland and multicellular hepatic necrosis.

Carglumic acid tested negative in the submitted genotoxicity tests (Ames mutagenicity test, *in vitro* chromosome aberration assay in human peripheral blood lymphocytes, and the *in vivo* micronucleus assay in rats).

In reproductive toxicology studies, no drug-related fetal malformations were observed. Female rats treated orally with 2000 mg/kg/day carglumic acid starting at 2 weeks prior to mating through the end of fetal organogenesis (gestation day 17) exhibited decreased body weight gain. Pregnant female rabbits receiving oral doses of carglumic acid at 1000 mg/kg/day during organogenesis (day 6 to 18 of gestation) showed decreases in body weight gain, food consumption, and uterine weight. In a peri-/post-natal developmental study, mated female rats were given 0, 500, or 2000 mg/kg/day carglumic acid from gestation day 6 to post-partum day 21. At 2000 mg/kg/day, hypersalivation and reduced body weight gains were noted during pregnancy, and maternal death occurred during gestation and lactation (2/20 pregnant females died on GD 18 and 1/18 lactating females died on day 17 postpartum). At this dose, higher mortality rates were noted in the pups during the first 4 days of lactation, with reduced body weight gains until the end of lactation. Decreased body weight of rat pups during lactation and at weaning were noted at 500 and 2000 mg/kg/day, compared to controls. In these offspring, no effects on physical and sexual development, neurobehavior and spontaneous locomotor activity, or mating, fertility, and gestation indices were noted at doses up to 2000 mg/kg/day.

Based on the recommended starting dose range for Carbaglu® (100-250 mg/kg/day), patients with NAGS deficiency will need to ingest a large number of tablets, which contain several excipients, to achieve a therapeutic effect (e.g., 30-75 tablets/day in 60-kg patient). However, the levels of excipient intake from Carbaglu® appear to be safe.

Hydantoin-5-propionic acid (HPA) and (b) (4) are impurities in the drug product. HPA is also a physiologic metabolite of histidine in humans. The proposed HPA limit of (b) (4) is lower than the ICH impurity qualification threshold (0.15%) for drug products, based on the maximum recommended dose of Carbaglu®. HPA was positive in the *in vitro* chromosomal aberration test, but was negative in the Ames test and *in vivo* micronucleus test. Given that HPA is a known endogenous substance, and that the positive genotoxicity finding was limited to the chromosomal aberration test, the proposed limit is considered to be acceptable.

(b) (4) is an impurity arising from an impurity (b) (4) in the starting material. The Sponsor's proposed limit of this impurity in commercial batches of drug substance is not more than (b) (4). The concentration of (b) (4) (b) (4) in the drug used in nonclinical studies was not measured, and no nonclinical studies have been conducted to examine the potential toxicity of (b) (b) (4). Because the level of (b) (4) was undetectable in the HPLC assay with a detection limit of 0.10%, it can be concluded that this impurity in the drug product that has been administered in humans was less than (b) (4). Thus, we concur with the recommendation of Dr.

Martin Haber, the Review Chemist, that the limit for (b) (4) (b) (4) in the drug product should be not more than (b) (4).

B. Pharmacologic activity

Carglumic acid (N-carbamoyl-L-glutamic acid) is a structural analog of N-acetyl-L-glutamate (NAG), which is an obligatory allosteric activator of mitochondrial carbamoyl phosphate synthetase 1 (CPS 1), the first enzyme of the urea cycle. NAG is synthesized by the enzyme N-acetylglutamate synthase (NAGS). In the absence of NAGS, NAG is not produced and plasma levels of ammonia are elevated due to impaired function of the urea cycle. Carglumic acid passes into the mitochondria of hepatocytes where it activates CPS 1. It is a weaker *in vitro* activator of CPS 1 than the naturally occurring activator NAG; however, carglumic acid stimulates the enzyme CPS 1 with greater efficiency than NAG *in vivo*. NAGS deficiency is one of the most severe and rarest of the hereditary urea cycle disorders. Carbaglu® was developed for the treatment of hyperammonemia associated with NAGS deficiency.

C. Nonclinical safety issues

Toxicology studies of carglumic acid were performed only in rats. For drug development, the Agency routinely requires toxicity studies in one rodent and one nonrodent species to assure safety in clinical studies and to support market approval. These studies include histopathologic evaluation of all tissues, which allows for a detailed evaluation of drug-induced toxicity. Thus, animal toxicity studies provide safety information that cannot be acquired from clinical studies. The use of two species in toxicity testing, as compared to a single species, is expected to provide greater sensitivity for the detection of potential toxic responses in humans. Because of the proposed chronic indication of Carbaglu, chronic toxicity studies in a rodent species (6-month treatment) and a nonrodent species (9-month treatment) are needed. However, the Applicant has not conducted any toxicity study in a nonrodent species.

In a pre-IND meeting on September 24, 2002, the Agency requested that a chronic toxicity study in a nonrodent species (in addition to a chronic rodent study) be conducted to support a NDA submission for Carbaglu. However, the Applicant submitted toxicity studies conducted in rats only in its IND application, and the clinical testing of Carbaglu was allowed to proceed without toxicity studies in a nonrodent species. In a pre-NDA meeting on April 28, 2004, the Sponsor disputed the need for a chronic toxicity study in a nonrodent species, citing a "lack of toxicity observed in a relevant species" as the reason for not conducting the study. The Sponsor also cited a lack of adverse events in humans after chronic dosing, as another justification. However, histopathologic examination in nonclinical toxicity studies provides highly detailed information

about potential drug-induced lesions, and such information can never be obtained from human studies. In a meeting on September 26, 2008, the FDA disagreed with the Sponsor's reasoning, and the deficiency was communicated again to the Sponsor. In this NDA submission, the Sponsor provided a comparison of carglumic acid metabolic profiles in humans, rats, dogs, and monkeys, in an effort to justify the use of rats as the only species in toxicology studies. However, the results of the cross-species comparison provide no justification for limiting toxicity testing to rats only.

Adding to the importance of a nonrodent toxicity study is the fact that well-controlled clinical studies were not provided in the current NDA submission. Furthermore, the clinical safety database for long-term use of Carbaglu® is based on a very small number of NAGS patients (n=26). Therefore, toxicity evaluation in a second (nonrodent) species will provide needed information for the safety profile.

Another nonclinical issue is the absence of carcinogenicity studies. Evaluation of the carcinogenic potential of carglumic acid is needed because of the chronic indication. To this end, the Sponsor has committed to conduct a two-year carcinogenicity study in a single species following approval for marketing.

Thus, the Applicant should be required to conduct a chronic (9-month) toxicity study in a nonrodent species and a two-year carcinogenicity study in a single species. Given that the proposed indication for Carbaglu, NAGS deficiency, is a life-threatening condition with no effective therapy available, it is acceptable for both of these studies to be conducted post-approval.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

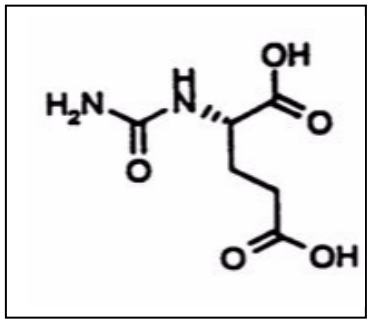
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22,562
Review number: 1
Sequence number/date/type of submission: 000
Information to sponsor: Yes (x) No ( )
Sponsor and/or agent: Orphan Europe
Paris, France
Manufacturer for drug substance: (b) (4)

Reviewer name: Yuk-Chow Ng, Ph.D.
Division name: Division of Gastroenterology Products
HFD #: 180
Review completion date: 02/17/2010

Drug:

Trade name: Carbaglu®
Generic name: Carglumic acid
Code name: OE 312
Chemical name: N-carbamoyl-L-glutamic acid
CAS registry number: 1188-38-1
Molecular formula/molecular weight: $C_6H_{10}N_2O_5$ / 190.06
Structure:



Relevant INDs/NDAs/DMFs:

IND 61,265 (Carbaglu® for treatment of NAGS deficiency), Orphan Europe

Drug class: Carbamoyl Phosphate Synthetase 1 activator

Intended clinical population: Patients with hyperammonemia due to N-acetylglutamate synthase deficiency

Clinical formulation:

Composition for a bar-shaped tablet, size 18x6.0 mm, with 3 break-marks both sides ((b) (4) "C" are engraved on one side of each tablet of Carbaglu tablet), containing 200 mg carglumic acid:

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)

Route of administration: Oral

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

Nonclinical Study	Testing Laboratory	Report #	Lot #	Page
PHARMACOLOGY				
Comparison of three different preparations of N-carbamoyl-L-glutamic acid in their ability to activate carbamoyl phosphate synthetase 1	(b) (4)	N/A	KLA 1028B, 040307102, and (b) (4) (unspecified)	18
SAFETY PHARMACOLOGY				
Behavioral Irwin test and effect on body temperature following single oral administration in rats of both sexes	(b) (4)	20000366 P	05031001P38	19
Evaluation of effect on respiration in the unrestrained conscious rat following single oral administration of both sexes	(b) (4)	20000367 P	05031001P30	20
Evaluation of effects on blood pressure, heart rate and electrocardiogram after single oral dosing in conscious dogs	(b) (4)	20000429 P	05031001P38	19
Evaluation of effect on cardiac action potential in isolated canine purkinje fibers	(b) (4)	20010264PCEM	05031011P62, CBF 0101	19
PHARMACOKINETICS/ TOXICOKINETICS				
Biodisposition of ^{14}C-CGA after a single oral administration to male and female Sprague-Dawley rats	(b) (4)	026/01 005	2C-001207	21
ADME study of carglumic acid after oral and intravenous administration of carglumic acid labeled with ^{14}C and ^{13}C to dogs	(b) (4)	026/06 027	05911609P646 05911610P646 CFQ14918 Batch1	22
Evidence of N-Carbamyl-L-Glutamic acid metabolites in human and rat hepatocytes	(b) (4)	026/00 080	05030906P28	28
GENERAL TOXICOLOGY				
N-Carbamyl-L-glutamic acid – single dose toxicity study by the oral route in the rat (Limit test)	(b) (4)	800/005	KLA1028B	35
N-Carbamyl-L-glutamic acid – single dose toxicity study by the intravenous route in the	(b) (4)	800/008	KLA1028B	35

rat				
2-week toxicity study by oral route (gavage) in newborn rats	(b) (4)	20329TSR	05031001P39	36
26-week toxicity study by oral route (gavage) in young rats	(b) (4)	20330TCR	05031001P38, 05031001P39, 05031002P42, 08031010P51, 08031010P52	46
GENETIC TOXICOLOGY				
Salmonella typhimurium/N-Carbamyl-L-glutamic acid-mammalian microsone plate incorporation assay	(b) (4)	800/001	KLA1028B	57
Salmonella typhimurium / N-Carbamyl-L-glutamic acid – Bacterial reverse mutation test	(b) (4)	800-010	040307102	59
N-Carbamyl-L-glutamic acid – In vitro chromosome aberration test in human lymphocytes	(b) (4)	800-009	KLA1028B	61
Test for chromosome aberrations by in vitro human lymphocyte metaphase analysis with carbamyl glutamate	(b) (4)	IPL-R-980904	03030804P282	67
Test for chromosome aberrations by in vitro human lymphocyte metaphase analysis with carbamyl glutamate (assay with and without neutralization in the absence of S9 and 24-hour treatment	(b) (4)	IPL-R-981108	03030804P282	71
N-Carbamyl-L-glutamic acid – rat erythrocyte micronucleus test in bone marrow	(b) (4)	800/004	KLA1028B	73
Mutagenicity study using the micronucleus test in rat with N-Carbamyl-L-glutamic acid	(b) (4)	IPL-R990505	KLA1028B	78
Bone marrow micronucleus test after 4-week treatment by oral route (gavage) in rats	(b) (4)	20334 MAR	05031001P39 05031001P42	82
Mutagenicity test on bacteria using B.N. Ames's technique with hydantoin 5-propionic acid	(b) (4)	IPL-R-980804	03149806R21	69
Test for chromosome aberrations by in vitro human lymphocyte metaphase analysis with	(b) (4)	IPL-R-980906	03149806R21	86

hydantoin 5-propionic acid				
Mutagenicity test on bacteria using B.N. Ames's technique with Diaza-1,3-dione-2,4-carboxy-7-cycloheptane	(b) (4)	IPL-R-081001	JT-AC-T164	93
In vivo micronucleus test in mice with hydantoin 5-propionic acid	(b) (4)	IPL-R-081003	JT-AC-T151	96
REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY				
Effects of N-Carbamyl-L-glutamic acid on fertility and embryo-foetal development by oral route (gavage) in female rats	(b) (4)	23288RSR	08031011P63	99
Effects of N-Carbamyl-L-glutamic acid on embryo-fetal development by oral route (gavage) in rabbits	(b) (4)	24241RSL	05031207P124 05031207P124, 05031212P142, 05031301P143	107
Effects of N-Carbamyl-L-glutamic acid on pre- and post-natal development by oral route (gavage) in rats	(b) (4)	24242RSR	05031112P106 05031207P123 05031207P124	130

2.6.2 PHARMACOLOGY

The following reviews and summaries are adapted from the Pharmacologist's reviews under IND 61,265 dated 8/20/2003, 5/3/2004, and 6/2/2005.

2.6.2.1 Brief summary

Carglumic acid (N-carbamoyl-L-glutamic acid) is a structural analog of N-acetyl-L-glutamate (NAG), which is an obligatory allosteric activator of mitochondrial carbamoyl phosphate synthetase 1 (CPS 1), the first enzyme of the urea cycle. NAG is synthesized by the enzyme N-acetylglutamate synthase (NAGS). In the absence of NAGS, NAG is not produced and plasma levels of ammonia are elevated due to impaired function of the urea cycle. NAGS deficiency is one of the most severe and rarest of the hereditary urea cycle disorders.

Carglumic acid passes into the mitochondria of hepatocytes where it activates CPS 1. Carglumic acid is a weaker *in vitro* activator of CPS 1 than the naturally occurring activator NAG; however, it stimulates CPS 1 with greater efficiency than NAG *in vivo*. Carglumic acid increased survival rate in rats following a lethal dose of ammonium acetate. Similarly, in partially hepatectomized rats, treatment with carglumic acid prior to the injection of ammonium acetate protected rats from ammonia intoxication.

No significant effects on blood pressure, heart rate, and ECG have been found in conscious dogs after single oral doses up to 1000 mg/kg carglumic acid. The drug has no significant effects on cardiac action potential in isolated canine purkinje fibers at up to

$1x10^{-4}$M. Carglumic acid also has no significant effects on CNS and respiratory functions in rats at doses up to 1000 mg/kg.

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

In vitro and *in vivo* studies in ureotelic animals (mostly in rats and mice) have shown evidence that carglumic acid is able to pass into the mitochondria of hepatocytes where it activates CPS I. Even if carglumic acid is shown to be an *in vitro* activator of CPS I quite weaker than the naturally occurring activator NAG, it is clearly demonstrated *in vivo* that carglumic acid stimulates the enzyme CPS with more efficiency than NAG. When injected to mice, radiolabeled N-acetyl-(^{14}C)-L-glutamate cannot be detected in liver mitochondria as opposed to N-carbamoyl-(^{14}C)-L-glutamate (Rubio, 1981). This could be explained in particular by a higher permeability of the mitochondria membrane to carglumic acid than to NAG (Meijer, 1982) but also by a greater resistance of carglumic acid to hydrolysis by aminoacylase present in the cytosol (Kim et al., 1972).

Drug activity related to proposed indication:

The following summaries were provided by the Sponsor; the information is from two published studies by Kim S et al. (Proc. Natl Acad. Sci USA, 69: 3503, 1972 and Biochem Biophys Res Comm 248: 391,1998).

a. The pharmacological activity of the drug was examined in rats given a lethal dose of ammonium acetate (10.8 mmol/l). Following an IP injection of 1-4 mmol/l of carglumic acid, a 61-76% survival rate was observed in rats. In contrast, NAG (4 mmol/l) produced protection of only 2%.

b. In the 70% partially hepatectomized rats, when animals were treated with carglumic acid prior to the injection of 3.4 mmol/l of ammonium acetate, the animals were protected from ammonia intoxication (ammonia levels were decreased to 225 vs 278 µmol/L in controls, $p<0.05$). The study showed that when the functional liver mass is significantly reduced, pretreatment with the drug alone was able to protect rats from ammonia intoxication.

ADDENDUM: The Sponsor compared three different preparations of carglumic acid (Batch KLA 1028B from (b) (4), Batch 040307102 from Orphan Europe, and a sample from the (b) (4)) and determined that the three powder preparations are equivalent on a powder weight basis in their ability to activate carbamoyl phosphate synthetase 1.

2.6.2.3 Secondary pharmacodynamics

No studies were submitted.

2.6.2.4 Safety pharmacology

Effects of carglumic acid on blood pressure, heart rate, and ECG after single oral dose in conscious dogs (Study no. 20000429 P, (b) (4))

Dogs (3M +3F) previously instrumented with telemetric transmitters (under anesthesia with thiopental 20 mg/kg/day by i.v. and maintained with halothane) were administered with the vehicle (1% CMC, 10 ml/kg) or single doses of carglumic acid in a suspension by oral route at 250, 500, 1000 mg/kg. The telemetric measurements continued for 24 hours following dosing. Carglumic acid did not affect these parameters at doses up to 1000 mg/kg. At 250 mg/kg, a statistically significant decrease in the heart rate was observed (59 vs 78 beats/min in controls, $p<0.05$) which was noted at 20-24 hours post-dosing. Since this was not observed at higher doses, it was not attributable to carglumic acid. Plasma concentrations of N-carbamoyl-L-glutamic acid in the treated dogs (at 1000 mg/kg) ranged from 73.9-277 μg/ml.

Effects of carglumic acid on cardiac action potential in isolated canine Purkinje fibers (Study no. 20010264 PCEM, (b) (4))

Purkinje fiber preparations were obtained from 3 male dogs and perfused with carglumic acid at 10^{-4}-10^{-7} M (0.1% DMSO in Tyrode's solution). Action potential duration (at APD_{50}, APD_{70} and APD_{90}) was measured using DATAPAC acquisition under normal (60 ppm) and low (20 ppm) stimulation rate. Carglumic acid had no effect on action potential durations, while cisapride (the positive control at $3x10^{-7}$ M) induced an increase in duration of the action potential (under normal stimulation with carglumic acid, APD_{90} was 326-344 vs 424 ms with cisapride; under low stimulation with carglumic acid, APD_{90} was 417-443 ms vs 614 ms with cisapride).

Effects of carglumic acid on CNS system (Study no. 20000366 P, (b) (4))

The effects of carglumic acid on the CNS system and on body temperature were examined in rats after a single oral dose (8M+8F rats, using Irwin test). The rats were administered with the vehicle (1% CMC, 10 ml/kg) or single oral doses of carglumic acid in suspension at 250, 500, and 1000 mg/kg. The Irwin scores and measurements of body temperatures were performed at 1, 2, 3, 4, 6 and 24 hours post dosing. Carglumic acid had no effect on above parameters. In contrast, clonidine (3 mg/kg, a positive control) produced abnormal gait, decreases in spontaneous locomotor activity, piloerection, etc. in this study. In conclusion, carglumic acid had no effects on the CNS or body temperature in rats.

Effect of carglumic acid on respiration in rats

The rats were administered single doses of carglumic acid by oral route at 250, 500, and 1000 mg/kg. Respiratory parameters were measured up to 4 hours. The drug had no effect on respiratory parameters. In contrast carbamylcholine (30 mg/kg, a positive control) produced bronchoconstrictor effects. These data were presented only as a summary.

ADDENDUM: The respiratory safety study was submitted in this NDA application, and the following is a summary of the study.

Effect of carglumic acid on respiration in rats (Study no. 20000367 P, (b) (4))

The effects of carglumic acid on respiratory parameters were examined in unrestrained conscious rats (4/sex/group). Animals were administered single doses of carglumic acid by oral route (gavage) at 250, 500, and 1000 mg/kg. Respiratory parameters (respiratory rate, peak inspiratory and peak expiratory flows, inspiration and expiration times, airway resistance, tidal volume) were measured up to 4 hours after carglumic acid administration. Carglumic acid had no effect on any of the respiratory parameters being measured. By contrast, carbamylcholine (30 mg/kg, a positive control) produced statistically significant increases in respiratory rate, peak inspiratory flow, peak expiratory flow, inspiration time, tidal volume, and airway resistance, and a decrease in expiration time. In conclusion, carglumic acid did not have any significant effects on the respiratory functions in conscious rats under the testing conditions.

2.6.2.5 Pharmacodynamic drug interactions

No studies were submitted.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Not applicable.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

All except one of the pharmacokinetic and toxicokinetic studies have been reviewed under IND 61,265 by Dr. Indra Antonipillai (reviews dated 8/20/2003, 5/3/2004, and 6/2/2005). As requested by the Agency, the Sponsor has submitted an ADME study in dogs. The following is a compilation of the three previous reviews, as well as a review of the newly submitted ADME study in dogs.

2.6.4.1 Brief summary

After oral administration, maximal concentrations of carglumic acid in rats and dogs were reached between 2 to 3 hours, and C_{max} values were similar after identical doses of carglumic acid. In humans, T_{max} was reached between 1.5 to 4 hours and plasma levels were lower than that of rats and dogs, on a dose-adjusted basis (3-4 µg/ml at 100 mg/kg carglumic acid in humans vs 52-112 µg/ml at 500 mg/kg in rats and dogs). The AUC in rats was lower at the higher dose than at the lower dose. In pregnant rabbits and rats, AUC values were also lower at the higher dose and at later stage of pregnancy. These results suggest an auto-induction of carglumic acid metabolism. In patients with hyperammonemia, the exposure levels were similar to that of the healthy volunteers after comparable doses of carglumic acid.

[^{14}C]Carglumic acid was widely distributed in rats; cecum had the highest levels of radioactivity, followed by small intestine membrane, kidneys, liver, mesenteric lymph nodes, cartilage, pancreas, and salivary glands.

In *in vitro* metabolism studies, there was no detection of the potential carglumic acid metabolites hydantoin-5-propionic acid (HPA) and diaza-1,3-dione-2,4-carboxy-7-cycloheptane (diaza-cycloheptane). In rats, dogs, and humans, most of the carglumic acid remains unchanged after oral dosing, and a small fraction of carglumic acid (up to 9%) is metabolized and excreted as CO_2. Studies in rats and dogs failed to detect HPA, diaza cycloheptane, or L-glutamic acid (another potential metabolite). In humans, two peaks of radioactivity were detected in plasma after a single oral administration of radiolabeled carglumic acid. The first peak matched well with the unchanged carglumic acid; the second peak, with a T_{max} of 36-48 hours, is speculated to represent the systemic circulation of water-soluble metabolites before their pulmonary elimination. Low levels of HPA and L-glutamic acid were present in human feces following oral administration of carglumic acid. However, neither HPA nor L-glutamic acid is a significant metabolite of carglumic acid, and its presence in feces appears to be limited to the lumen of the digestive tract.

In rats and dogs, the plasma concentration showed biphasic elimination with an initial rapid elimination phase (70%) within 12 hours post-dose, followed by a slower phase over the period of 12-96 hours. The major route of elimination in rats and dogs was the urinary route (50%) and that in humans was the fecal route (approximately 70%).

2.6.4.2 Methods of Analysis
See under individual study reviews.

2.6.4.3 Absorption

Single Dose Studies

In a single oral (gavage) dose study in rats, the plasma concentration was 60-70 µg/ml at 500 mg/kg of carglumic acid.

In another study, bio-disposition of ^{14}C-CGA after oral administration to male and female SD rats was examined. Male and female rats received the labeled drug plus 500 mg/kg carglumic acid by oral route in 1% CMC. Blood and plasma PK, excretion of radioactivity via urinary, fecal, or pulmonary routes, and metabolism in urine and plasma were studied. The results are shown in the table below (taken from the Sponsor).

Table 7.1-1.: Concentrations in blood and plasma (nCi./g and µg eq./g) following a single (500 mg/kg) oral dose of ^{14}C-CGA to male or female Sprague-Dawley rats

Rat N°	Time (hours post-dose)	Conc. (nCi/g)		Conc. (µg Eq./g)		(Blood/plasma) concentration ratio
		Blood	Plasma	Blood	Plasma	
M1 - M2 (mean)	3	14.4	23.2	45.5	73.4	0.6
F1 - F2 (mean)		11.0	16.4	34.8	51.9	0.7
M3 - M4 (mean)	8	6.0	8.5	19.0	26.9	0.7
F3 - F4 (mean)		5.1	6.5	16.1	20.6	0.8
M2	24				(b) (4)	1.0
F2						1.1
M3	48					1.2
F3						1.3
M4	96					1.8
F4						1.5

The maximal radioactivity in plasma was noted at 3 hours post dosing and the concentrations were 73.4 µg·eq/ml in males and 51.9 µg·eq/ml in females. By 96 hours the levels in plasma were about 2 µg·eq/ml for both sexes. The plasma concentration showed biphasic elimination with a first rapid elimination (70%) within 0-12 hours and the rest up to 7 days post–dosing.

In a single dose study in dogs (3M+3F), after oral administration of carglumic acid at 1000 mg/kg (from the Safety Pharmacology study), the plasma concentrations were 74-277 µg/ml.

ADME study of carglumic acid after oral and intravenous administration of carglumic acid labeled with ^{14}C and ^{13}C to dogs (Study no. 026/06 027, (b) (4) (b) (4))

Note: The Sponsor submitted this study in response to the Agency's request for a comparison of cross species metabolic profiles, including a non-rodent species.

Methods:

Three young male and three young female beagle dogs, with a body weight ranging from 5.4 to 6.0 kg (first phase) and from 5.8 to 6.9 kg (second phase), were included in this study. The six dogs were treated orally with radioactive [^{14}C]carglumic acid and nonradioactive [^{13}C]carglumic acid at 504.8 mg/kg in carboxymethyl cellulose (1%). The mean radioactive dose administered was 1918.6 kBq/kg. After a wash-out period of 7 days, the same dogs were treated intravenously with ^{14}C- and ^{13}C-carglumic acid at 497.4 mg/kg in purified water. The mean radioactive dose administered was 1888.1 kBq/kg.

Urine fractions were collected over 0-2, 2-4, 4-6, 6-8, 8-12 and 12-24 hr. Feces were collected over 0-4, 4-8, 8-12, and 12-24 hr. Cage washes were collected over 0-8 and 8-24 hr. Urine, feces, and cage washes were then collected once daily over a 168-hr period. CO_2 excreted was collected for 5 minutes at 1, 2, 3, 4, 6, 8, 12, 24, 48, and 72 hr after oral administration, and at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 hour after intravenous infusion. Blood was collected at predose, 5, 10, 15 min, and 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, 120, and 168 hr after intravenous infusion. After oral administration, blood was collected at predose, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, and 168 h.

Results:
One animal vomited during the first two hours after oral administration, and consequently, the animal was excluded from the excretion mass balance analysis. In the other animals, frequent diarrhea was observed after treatment. In two animals, the percent recovery of radioactivity in urine and cage washes was overestimated. One animal vomited about 3.5 hours after intravenous administration. Otherwise, no clinical adverse events were observed.

Plasma kinetic parameters for radioacitivity are shown in the table below.

	T_{max} (hour)		**C_{max} (µg/ml)**	
	M	F	M	F
Oral	2.0	2.5	112	111
Intravenous	0.25	0.25	2167	2098

For the oral route, the recovery of the radioactive dose was 89.1% in males and 86.1% in females. For the i.v. route, the recovery was 90.5% in males and 89.0% in females. After oral administration, most of the elimination of radioactivity occurred in the first 24-hours post-dose, with about 80% of the radioactive dose (81.9% for male animals and 78.0% for female animals) being recovered within that period. The major route of elimination was urinary, with 50% of the radioactive dose in male animals and 42% in female animals recovered after 168 hours. Excretion in the feces accounted for 18% of the dose in male animals and 35.2% in female animals within 168 hours. Cages washes contained 21.3% of the dose in male animals and 9.3% in female animals.

A small quantity of the drug-related material was excreted by the respiratory route: from 1.09% to 1.52% and from 0.40% to 0.70% of the orally and intravenously administered

doses, respectively, was found in the expired CO_2. Metabolism of [^{13}C]carglumic acid did not lead to formation of [^{13}C]hydantoin-5-propionic acid, [^{13}C]diaza- cycloheptane, or [^{13}C]L-glutamic acid in the blood, urine, or feces. No differences were observed between male and female animals in the mass balance excretion or pharmacokinetic study.

Summary: In dogs, T_{max} and C_{max} after an oral dose of 500 mg/kg carglumic acid were 2.0 to 2.5 hours and 111-112 µg/ml, respectively. The major route of elimination was the urinary route, with lesser excretion from the fecal route. A small quantity of the drug was excreted by the respiratory route as expired CO_2. Metabolism of carglumic acid did not lead to detectable levels of hydantoin-5-propionic acid, diaza-cycloheptane, or L-glutamic acid, which are all considered as potential metabolites of carglumic acid. It is noted that HPA and L-glutamic acid were identified as metabolites in humans, but were detected only in feces. Thus, the metabolic profile of carglumic acid in dogs appears to be more similar to that of rats than that of humans.

Multiple Dose Studies

Following oral dosing of 250 mg/kg/day carglumic acid in a 2-week toxicity study in rat pups, maximal plasma concentrations were noted at 4 hours post-dose and the levels were 39-50 µg/ml in the males and females.

In a 26-week toxicity study in rats, the maximal plasma concentrations were achieved 3 hours post-dose in males and at 2 hours post-dose in females. No significant sex differences in C_{max} or AUC were noted in week 1 between males and females. AUC (but not C_{max}) values generally increased in a less than dose proportional manner. There was slight accumulation of carglumic acid with time in males at 500 mg/kg/day in week 26 vs week 1 (477.7 µg•h/ml on week 1 vs 891.1 µg•h/ml on week 26), and there was a 3-fold accumulation seen in females (323 µg•h/ml on week 1 vs 1009 µg•h/ml on week 26). At 500 and 1000 mg/kg/day, AUC values in males at week 26 were 891 and 716 µg•h/ml, respectively, and in females were 1009 and 810 µg•h/ml, respectively. The lower AUC values at the higher dose levels suggest auto-induction of metabolism of carglumic acid. Urine drug concentrations were not significantly different between sexes (131-184 mg/24 hr in males and 75-129 mg/24 hr in females).

In pregnant rabbits, the AUC values of the drug were 50% lower on day 18 (72 and 126 µg•h/ml at 250 and 1000 mg/kg/day, respectively) than on day 6 (176 and 654 µg•h/ml at 250 and 1000 mg/kg/day, respectively), suggesting autoinduction of drug metabolism or modification of its absorption. Also, in pregnant rats, after oral administration of carglumic acid to mated females from day 6 of gestation to day 21 of post-partum, the plasma drug concentrations were lower at the higher dose at sacrifice (70 and 18 µg/ml at 500 and 2000 mg/kg/day, respectively), again suggesting autoinduction of drug metabolism (see Segment III Reproduction study in rats for details).

2.6.4.4 Distribution

Distribution was evaluated from an *in vivo* study in rats (1M+1F) given a single dose of 500 mg/kg ^{14}C-carglumic acid, using microscopic autoradiography and quantitative radioluminography at 3 hours and 96 hours post-dose. The radioactivity was widely distributed. When plasma radioactivity reached its peak value at 3 hours post-dose (52-73 µg·eq/g), cecum had the highest activity (12,369 µg·eq/g), followed by small intestine membrane (6288 µg·eq/g), kidneys (1403 µg·eq/g), liver (582 µg·eq/g), penis (430 µg·eq/g), mesenteric lymph nodes (162 µg·eq/g), cartilage (116 µg·eq/g), pancreas (96 µg·eq/g), and salivary glands (95 µg·eq/g). Significant level of radioactivity was still noted in several tissues, including kidneys and liver (up to 0.025%), at 96 hours post-dose. The data are shown in tables below:

Table. Tissue distribution at 3 hours post-dose.

Tissue distribution of ^{14}C-CGA after a single oral (500 mg/kg) administration in Sprague-Dawley rats (results expressed as µg equ./g of tissue)

	3 hours post-oral administration	
Organ / tissue / fluid	M1 (male)	F1 (female)
adrenal gland	39.223	108.812
artery wall	BQL	BQL
blood (heart cavity)	65.477	42.070
bone marrow (femur)	50.926	43.335
bone marrow (vertebrae)	53.773	31.631
brown fat	63.895	58.518
caecum	*12369.402*	*17723.941*
cartilage (femur)	116.403	87.619
cartilage (vertebrae)	37.641	29.101
cerebellum	6.959	7.275
cerebrum	7.908	7.908
choroid plexus	BQL	BQL
epididymal fat	8.540	-
epididymis	39.855	-
eye	BQL	BQL
Harderian glands	68.007	80.343
hypophysis	55.355	33.845
kidney (cortex)	745.232	501.355
kidney (inner cortex)	1403.162	744.283
kidney (medulla)	315.047	662.991
liver	581.699	473.520
lungs	58.518	39.855
mesenteric lymph nodes	162.901	74.017
muscle (skeletal)	17.081	10.122
myocardium	37.325	18.979
ovary	-	58.834
pancreas	95.526	57.885
penis	430.185	-
perirenal fat	54.722	10.122
salivary glands	94.577	65.793
seminal membrane	BQL	-
seminal vesicles	65.793	-
skin-hair	66.109	43.967
small intestine contents	1226.027	560.190
small intestine membrane	*6287.977*	2224.942
spinal cord	7.275	7.908
spleen	45.865	36.376
stomach contents	2554.540	2545.367
stomach membrane	NQ	NQ
stomach mucosa	79.394	40.804
sublingual glands	65.477	46.182
testis	22.142	-
thymus	31.631	35.743
thyroid	61.681	36.376
tongue	45.233	29.417
uveal tract	29.417	22.775
white fat	41.437	36.692

(BQL): Below the Quantitation Limit (< 2.214 µg equ./g)
(*ital*): extrapolated value (> 5848.935 µg equ./g) (NQ): Not Quantified

Table. Tissue distribution at 96 hours post-dose

Tissue distribution of ^{14}C-CGA after a single oral (500 mg/kg) administration in Sprague-Dawley rats (results expressed as µg equ./g of tissue)

	96 hours post-oral administration	
Organ / tissue / fluid	M4 (male)	F4 (female)
adrenal gland	9.806	11.387
artery wall	BQL	BQL
blood (heart cavity)	3.479	4.428
bone marrow (femur)	7.275	8.224
bone marrow (vertebrae)	6.010	6.326
brown fat	5.377	10.755
caecum	BQL	BQL
cartilage (femur)	10.438	BQL
cartilage (vertebrae)	BQL	BQL
cerebellum	2.214	3.479
cerebrum	2.531	2.531
choroid plexus	BQL	BQL
epididymal fat	8.857	-
epididymis	5.061	-
eye	BQL	BQL
Harderian glands	10.755	12.653
hypophysis	6.643	3.796
kidney (cortex)	17.714	24.989
kidney (inner cortex)	BQL	55.355
kidney (medulla)	43.967	4.112
liver	26.254	33.213
lungs	3.796	3.796
mesenteric lymph nodes	10.755	14.867
muscle (skeletal)	3.163	BQL
myocardium	4.745	3.163
ovary	-	12.653
pancreas	6.326	7.592
penis	BQL	-
perirenal fat	8.224	11.071
salivary glands	7.275	8.224
seminal membrane	BQL	-
seminal vesicles	8.857	-
skin-hair	9.173	3.796
small intestine contents	BQL	BQL
small intestine membrane	7.592	BQL
spinal cord	3.479	3.163
spleen	7.908	7.592
stomach contents	BQL	BQL
stomach membrane	BQL	BQL
stomach mucosa	7.592	7.908
sublingual glands	BQL	BQL
testis	BQL	-
thymus	10.438	10.438
thyroid	BQL	BQL
tongue	BQL	4.428
uveal tract	BQL	BQL
white fat	11.071	9.489

(BQL): Below the Quantitation Limit (< 2.214 µg equ./g)

2.6.4.5 Metabolism

The metabolism of carglumic acid has been examined *in vitro* in cultured hepatocytes from rats and humans (from 2 donors of rats or male human subjects). Two concentrations of carglumic acid, 25 and 250 µM, were evaluated and no metabolites were observed using mass spectrometry or LC coupled to radioactivity.

9.1. *In vitro* (report 10)

The metabolism of carglumic acid has been investigated in vitro in human and rat hepatocytes. Cultured hepatocytes were obtained from 2 donors of each species (male Sprague-Dawley rats and male and female subjects with liver metastasis) and the cells were kept frozen until required.
Two concentrations, 25 µM and 250 µM, of the ^{14}C labelled parent drug were tested. The viability of the cells, throughout the kinetic studies involving a 24-hour incubation period, were confirmed by measuring the activity of a number of enzymes, EROD (CYP1A), PROD (CYP2B), deethylated phenacetin (CYP1A2) and nifedipine oxidase (CYP3A4), using Trypan blue and carrying out a morphological examination. As far as the analysis was concerned, separation was performed by liquid chromatography coupled to, in one case, a radioactivity detector and, in the other, a mass spectrometer. The formation of any metabolites carrying one or more ^{14}C atoms from the glutamic acid moiety could be followed by radioactivity detection. Moreover, any metabolite which might have corresponded to any possible internal cyclisation product, namely hydantoin-5-propionic acid and N-carbamyl pyroglutamic acid as heterocycles at 5 atoms, as well as diaza-1,3-dione-2, 4-carboxy-7-cycloheptane as heterocycle at 7 atoms, could be identified specifically by LC/MS or LC/MS-MS (figure 1). The fate of the parent drug was also investigated at the same time.
The results all agreed and allowed the conclusion that no metabolites were produced, at least in quantities above the limits of detection of each of the 2 methods used, LC coupled to radioactivity detection and LC coupled to mass detection. The same conclusions were reached in rat and man (figure 17).

Addendum: Figure 17 is not presented in this review.

IN VIVO METABOLISM OF CARGLUMIC ACID

9.2. In vivo

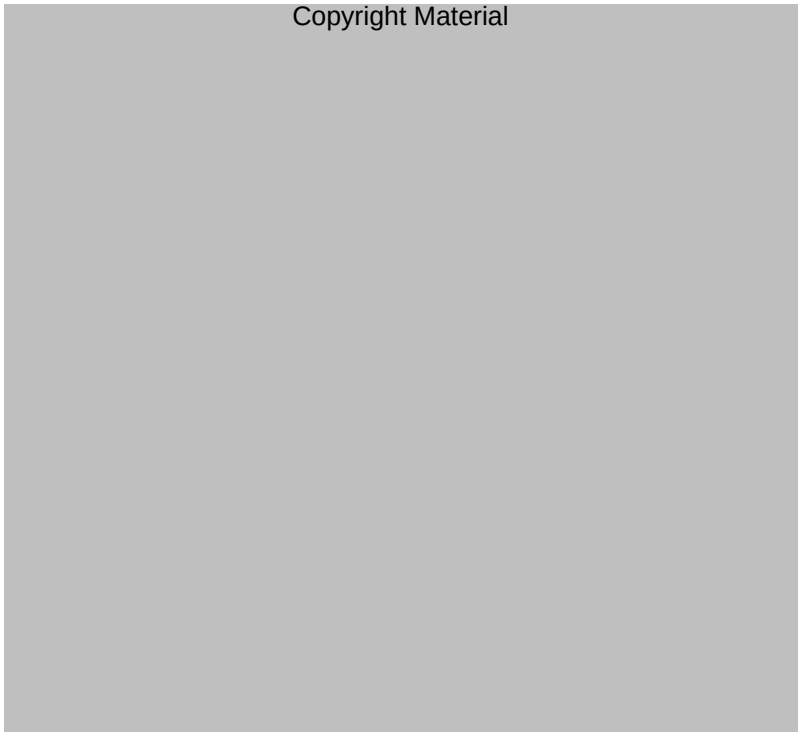
9.2.1. In the Rat (report 11)
After a single dose of 500 mg/kg radiolabelled carglumic acid, the *in vivo* metabolism of carglumic acid was investigated by LC coupled to radioactivity detection / UV detection.
In the first 24-hour urine collections, only one peak of radioactivity corresponding to the parent compound carglumic acid was evidenced (Figure 18).
In this study, about 9% of the total radioactivity was eliminated as expired labelled CO_2 rapidly within the first 24 hours post dose (6.9 % within the first 11 hours). No intermediate metabolites could be evidenced during that period but as extrapolated in section 4.2.1, radioactivity concentrations tend to be slightly higher than the expected parent compound

concentrations at the end of the first elimination phase, 12 hours post dose, and even more significantly higher at 24 hours. Therefore, the formation of metabolites circulating in blood before being eliminated as CO_2 is highly probable.

Addendum: Figure 18 is not presented in this review.

Formation of Impurities or Potential Metabolites from Carglumic Acid

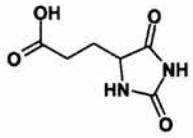
The Sponsor indicated that hydantoin-5-propionic acid (HPA) is a major impurity present in batches of carglumic acid. In addition, HPA is also an endogenous compound identified in some animal species and in humans. In monkeys, after radiolabeled histidine administration, glutamine and HPA was identified. HPA can be formed by the following pathway:



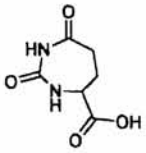
In addition, the Sponsor illustrates in the figure below the possible by-products of carglumic acid that form by internal cyclization before drug administration or *in vivo*:

Figure 1: Carglumic acid potential by-products by internal cyclisation

Most thermodynamically stable cyclised by-products

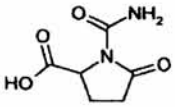


Hydantoin-5-propionic acid (HPA)



diaza-1,3-dione-2,4-carboxy-7-cycloheptane

Potential other by-product (never detected in pharmaceutical grade carglumic acid)



N-Carbamyl pyroglutamic acid

Thus, HPA is an impurity in carglumic acid preparations, and it can be formed by cyclization of carglumic acid, and by catabolism of histidine.

Metabolism Study in Dogs

In the newly submitted ADME study in dogs, after oral and i.v. administration of ^{13}C- and ^{14}C-carglumic acid (500 mg/kg), a small quantity of drug-related material was excreted by the respiratory route; 1.09% to 1.52% from orally administered dose and 0.40% to 0.70% from intravenously administered dose was found in the expired CO_2. Metabolism of [^{13}C]carglumic acid did not lead to formation of [^{13}C]hydantoin-5-propionic acid, [^{13}C]diaza-cycloheptane, or [^{13}C]L-glutamic acid, which are all considered as potential metabolites of carglumic acid (see study reviewed in section 2.6.4.3 for details).

2.6.4.6 Excretion

When [^{14}C]carglumic acid was administered orally (500 mg/kg) to rats, 40% of the radioactivity was found in the urine (49% in males, 32% in females) while 22% was recovered in feces (16% in males, 29% in females) at 12 hours post-dose. In the expired CO_2, 7% of radioactivity was recovered. Thus, elimination was rapid with 70% of the radioactivity excreted within 0-12 hours post-dose. After 12 hours, the radioactivity continued to be excreted through 7 days post-dose, when 0.25% was excreted in CO_2. The total radioactivity excreted was 90% within 24 hours and 97% at 96 hours. The remaining radioactivity was measured in the liver and kidneys, where 3% of the administered dose was recovered.

Table. Excretion in rats after oral dosing

Time interval	% of administered dose (mean)			% of cumulated excretion (mean)		
(h)	Urine + c.w.	Faeces	CO_2	Urine + c.w.	Faeces	CO_2
0 - 12 *	40.252	22.487	6.919			
12 - 24 **	6.376	12.573	0.994	46.628	35.060	7.913
24 - 48	2.133	2.061	0.613	48.761	37.121	8.526
48 - 72	0.481	0.057	0.269	49.242	37.180	8.795
72 - 96	1.496	0.021	-	50.738	37.201	

- : no measurement
* : 0 - 11 hours for ^{14}C-CO_2
** : 11 - 24 hours for ^{14}C-CO_2

Excretion Study in Dogs

In the newly submitted ADME study in dogs, after oral administration of ^{13}C- and ^{14}C-carglumic acid (500 mg/kg), most of the elimination of radioactivity occurred in the first 24 hours post-dose, with about 80% of the radioactive dose (81.9% for male animals and 78.0% for female animals) being recovered within that period. The major route of elimination was urinary, with 50% and 42% of the radioactive dose in males and females, respectively, recovered at 168 hours. Excretion in the feces accounted for 18% of the dose in males and 35.2% in females within 168 hours (see study reviewed in section 2.6.4.3 for details).

2.6.4.7 Pharmacokinetic drug interactions

No studies were submitted.

2.6.4.8 Other Pharmacokinetic Studies

No studies were submitted.

2.6.4.9 Discussion and Conclusions

In single oral dose studies, the plasma concentration of carglumic acid was 60 µg/ml in rats (at 500 mg/kg) and 74-277 µg/ml in dogs (at 1000 mg/kg). In the ADME study in dogs, after an oral dose of 500 mg/kg, the T_{max} was 2 hours for males and 2.5 hours for females, and C_{max} was 112 µg/ml for males and 111 µg/ml for females.

In a multiple dose study in rats (26-week study), after oral doses of 500 mg/kg/day and 1000 mg/kg/day, the maximal plasma concentration (69-104 µg/ml) was reached 2-3 hour post-dose; no significant sex-related differences in C_{max} or AUC were noted in week 1. At week 26, there was a higher accumulation of carglumic acid in females (3-fold) than in males (about 2-fold) at 500 mg/kg/day. The differences in accumulation between males and females were smaller at 1000 mg/kg/day. The AUC was lower at the higher dose than at the lower dose, which may suggest auto-induction of metabolism of carglumic acid. In pregnant rabbits and rats, AUC vales were also lower at the higher dose and at later stage of pregnancy, again suggesting auto-induction of carglumic acid metabolism.

In healthy volunteers, a single oral administration of 100 mg/kg radiolabeled carglumic acid produced two peaks of radioactivity in plasma. The first peak was observed at the same T_{max} as the parent compound (2.5 hours after drug administration) and up to 8-12 hours post-dose. The first peak appeared to correlate with unchanged carglumic acid. The second peak, which was not the parent compound, had much higher radioactivity, and appeared in blood and plasma with a T_{max} of 36-48 hours. The Sponsor speculated that the second peak is likely to represent the systemic circulation of water-soluble metabolites before their pulmonary elimination.

In a bio-disposition study using oral administration of 500 mg/kg [^{14}C]CGA to male and female SD rats, the maximal radioactivity in plasma was noted at 3 hours post-dose. The plasma concentration showed biphasic elimination with an initial rapid elimination phase (70%) within 0-12 hours, followed by a slower phase over the period of 12-96 hours. Elimination continued for up to 7 days post-dosing. By 96 hours the levels in plasma were about 2 µg·eq/ml for both sexes. Carglumic acid was widely distributed; cecum had the highest activity, followed by small intestine membrane, kidneys, liver, mesenteric lymph nodes, cartilage, pancreas, and salivary glands. Small amounts of radioactivity were noted in the spleen, thymus, brain, and testis. Low levels of radioactivity were still noted in several tissues at 96 hours post-dose, including kidneys and liver (up to 0.025%).

In an *in vitro* metabolism study of carglumic acid in rat and human hepatocytes, no metabolites were detected. Metabolism studies in rats and humans showed that after oral administration, approximately 95% of carglumic acid remains essentially unchanged. *In vivo* studies in rats and dogs failed to detect hydantoin-5-propionic acid, diaza-

cycloheptane, or L-glutamic acid, which are all considered as potential metabolites of carglumic acid. However, CO_2 was identified as a metabolite of carglumic acid in animals.

As seen in animals, humans metabolize a small percentage of carglumic acid to CO_2. Low levels of HPA and L-glutamic acid were present in human feces following oral administration of carglumic acid. HPA in humans is probably limited to the lumen of the digestive tract. Neither HPA nor L-glutamic acid is a significant metabolite of carglumic acid.

In rats, approximately 90% of the administrated [^{14}C]carglumic acid remained unchanged; about 40% of the radioactivity was recovered in the urine, 22% in feces, and 7% as expired CO_2. The elimination was rapid as 70% of the radioactivity was eliminated within 12 hours post-dose. In dogs, after oral administration of non-radioactive [^{13}C]carglumic acid and radioactive [^{14}C]carglumic acid (500 mg/kg), the major route of elimination was urinary (42-50%); 18-35% of the radioactivity was eliminated in feces. The elimination was also rapid, as 80% of the radioactive dose was recovered in the first 24 hours. About 1% of the orally administered dose was recovered in the expired CO_2.

Results from these studies indicate that the metabolism and excretion of carglumic acid in rats and dogs differ from that observed in humans. In humans, a greater percentage of carglumic acid was eliminated in the feces (approximately 70%) compared to rats and dogs (18-35%). Also, humans exhibit two peaks of radioactivity in plasma after oral administration of carglumic acid. The second peak, which has a T_{max} of 36-48 hours, may represent the systemic circulation of water-soluble metabolites before their pulmonary elimination. This peak was not observed in animals. Glutamic acid was identified as a metabolite in human feces, but was not detected in the animal studies. Finally, HPA was detected in human feces, and its presence appears to be limited to the lumen of the digestive tract.

2.6.4.10 Tables and figures

Not applicable.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Not applicable.

2.6.6 TOXICOLOGY

Parts of the following reviews and summaries are taken from the Pharmacologist's reviews under IND 61,265 dated 8/20/2003, 5/3/2004, and 6/2/2005.

2.6.6.1 Overall toxicology summary

In the 2-week oral toxicity study of carglumic acid in rat pups (4-day old), animals received carglumic acid (0, 250, 500, 1000, and 2000 mg/kg/day) from day 4 to day 21 postpartum. At the low dose, carglumic acid produced similar plasma concentration in males and females. All doses of carglumic acid produced mortalities, although only deaths at 2000 mg/kg/day were considered as drug related. In surviving animals, the incidence of clinical signs were of low incidence (e.g., swollen abdomen, wound in urogenital area, abnormally colored feces). Carglumic acid produced impairment of body weight gain at 1000 and 2000 mg/kg/day, decreased BUN at 1000 mg/kg/day, and decreased urinary pH at 250 mg/kg/day (6.5 vs 7.5 in control group). There were no gross pathological findings at up to 2000 mg/kg/day. The only significant histopathological finding in the main study animals was the dilated kidney pelvis in the 2000 mg/kg/day males (4/8 vs 1/8 in controls). Thus, the NOAEL (no observed adverse effect level) is considered to be 500 mg/kg/day, based on the reduced weight gain and the effect in kidneys at 1000 mg/kg/day.

In the 26-week oral toxicity study in juvenile (28-day old) rats (0, 500, and 1000 mg/kg/day, 10/sex/group), carglumic acid increased salivation at the high dose, and it significantly decreased urinary pH in the females at the high dose. The most frequent sign of toxicity was observed in kidney (interstitial mononuclear cell aggregation) at 500 and 1000 mg/kg/day. Target organs of toxicity at the high dose only were the Harderian gland (necrotizing inflammation) and liver (multicellular hepatic necrosis). An evaluation of fertility and mating performance in males at week 26 showed no effects. Immunotoxicity parameters were not affected. The tolerated dose of carglumic acid in the 26-week oral toxicity study in rats is considered to be 500 mg/kg/day. Based on AUC exposures, this dose is between 15 to 25 fold higher than the minimum recommended starting dose of 100 mg/kg/day (human exposure in healthy subjects at a single dose of 100 mg/kg was 21-23 µg·h/ml, and the AUC in rats was 323-564 µg·h/ml in week 1 at 500 mg/kg/day). However, the tolerated dose is only 2-fold higher than the maximum recommended starting dose of 250 mg/kg/day, based on a mg/kg comparison.

Genotoxicity studies showed that carglumic acid was negative in the Ames test and was not clastogenic in the *in vitro* chromosome aberration assay in human peripheral blood lymphocytes, when pH of the testing medium was adjusted. However, without pH adjustment, the results in the chromosomal aberration assay were positive. Carglumic acid containing HPA (0.1%) impurity was also negative in the *in vivo* micronucleus assay in rats. However, HPA was clastogenic in the chromosome aberration assay in lymphocytes. The totality of the genotoxicity studies indicates that carglumic acid lacks genotoxic activity, and that HPA and/or a reduction in pH were factors in the observed clastogenic effects of carglumic acid in some of the genotoxicity assays.

In the segment I/II rat fertility/embryo-fetal developmental study in rats, the NOAEL for maternal toxicity was considered to be 500 mg/kg/day, based on body weight decrements at 2000 mg/kg/day. No changes in mating, fertility (evaluated in females only), and gestation indices were noted. The maternal NOAEL is 5 times the minimum starting

dose in humans, based on a mg/kg comparison. Although significant increases in incomplete ossification of the supraoccipital bone were observed, the incidences at both doses were close to that of historical control data. Fetal NOAEL was considered to be 2000 mg/kg/day

In the segment II rabbit embryo-fetal developmental toxicity study, the NOAEL for maternal toxicity was considered to be 250 mg/kg/day, based on body weight and food consumption decrements at 1000 mg/kg/day (500 mg/kg/day was not tested). The maternal NOAEL is 2.5 times the minimum starting human dose, based on a mg/kg comparison. Fetal NOAEL was considered to be 1000 mg/kg/day, as no significant changes were observed in any of the parameters measured. This dose level is 10 times the minimum starting human dose.

In the Segment III peri-post natal developmental study in rats using dose levels of 0, 500, and 2000 mg/kg/day, 3 F0 female rats died at 2000 mg/kg/day, and hypersalivation and reduced bodyweight gains and food consumption during pregnancy were noted at the high dose. No significant effects on the gestation and delivery parameters were noted in the F0 generation. In the high-dose F1 pups, higher mortality rates were noted during the first 4 days of lactation, with reduced bodyweight gains in low-dose and high-dose pups until the end of lactation. Carglumic acid was excreted in the milk at significant levels. The lower body weight of F1 rats from treatment groups progressively returned to levels comparable to that of the controls at the end of the pre-mating period, except in high-dose males. Physical and sexual development were not affected in F1 pups, and results of the neurobehavioral and spontaneous locomotor activity tests were not significantly different from controls. There were also no significant effects on mating indices, fertility indices, and gestation indices and duration in the F1 pups. Taken together, the NOAEL was considered to be < 500 mg/kg/day for peri-post natal development in rats because of the impaired bodyweight gains in pups at 500 mg/kg/day.

2.6.6.2 Single-dose toxicity

Oral study: A single dose of 2806 mg/kg carglumic acid was administered to one set of 5 male + 5 female SD rats (by oral gavage in 1% aqueous CMC). Animals were observed for 14 days, and subjected to gross pathology. Carglumic acid was supposedly well tolerated, as no mortalities were observed. Also no clinical signs or changes in body weights or necropsy findings were observed.

IV study: In another study, 5 rats/sex/dose received a single dose of 239 mg/kg carglumic acid (by IV route in 0.9% saline). Animals were observed for 14 days, and subjected to gross pathology. Again carglumic acid was well tolerated, as no mortalities, clinical signs, changes in body weights, or necropsy findings were observed.

Therefore, single oral doses up to 2800 mg/kg and IV doses up to 239 mg/kg were well tolerated in acute studies in rats.

2.6.6.3 Repeat-dose toxicity

A 2-Week Oral (Gavage) Toxicity Study of Carglumic Acid in Newborn Rats (b) (4) study No. 20329 TSR)

Key study findings: Carglumic acid following 2-weeks oral administration in rat pups (0, 250, 500, 1000, 2000 mg/kg/day) produced deaths in all drug treated pups (0/16, 8/34, 4/16, 1/16, 39/39 at 0, 250, 500, 1000 and 2000 mg/kg/day respectively). Also, decreased urinary pH occurred in the 250 mg/kg/day group (6.5 vs 7.5 in controls, no data from higher dose levels), as observed in a 26-week toxicity study in young rats (5.2 vs 6.4 in controls). All doses of carglumic acid (except 1000 mg/kg/day) produced gross pathological findings in the stomach (brownish/whitish/yellowish contents in 0/16, 5/16, 3/16, 0/15, 6/15 respectively at 0, 250, 500, 1000, and 2000 mg/kg/day respectively). Some changes in organ weights and histopathological findings were observed at 1000 mg/kg/day. The mortality appears to be correlated to gross pathological findings in the stomach. No NOAEL could be established in this study, based on mortality seen even at the lowest dose of 250 mg/kg/day in pups. (see ADDENDUM for revised conclusions)

Study no: 20329 TSR

Volume #, and page #: 3.10, page 074

Conducting laboratory and location: (b) (4)

Date of study initiation: 8/22/2000

GLP compliance: yes

QA report: yes (X) no ( )

Drug lot #, and % purity: Batch # 05031001P39. This batch had HPA <0.02%.

Formulation/vehicle: 1% aqueous Carboxymethylcellulose (CMC). Carglumic acid was grounded as a fine powder and suspended in a vehicle 1% aqueous CMC, giving a concentration of 33.3, 66.7, 133, 267 mg/ml. It was stable up to 7 days at -20^{o}C. A constant dose of 7.5 ml/kg/day was given by gavage to pups.

Methods (unique aspects): For the main study, 80 pups (40 M+40F) from 10 litters were obtained from 13 adult mated females. For the satellite group, 36 pups (18M+18F) were obtained from 4 adult mated females. These pups were 4 days old, and approximately weighed 10 g for both sexes.

Dosing:
Species/strain: Rats Crl CD (SD) IGS, BR strain from (b) (4)
#/sex/group or time point (main study): 8/sex/dose at 250, 500, 1000, 2000 mg/kg/day.

Satellite groups used for toxicokinetic analysis: 9 males + 9 females were added for toxicokinetic (TK) analysis in the 250 and 2000 mg/kg/day groups. Also a satellite control group (8/sex/group) was added to the study. Satellite animals in TK groups were sacrificed at the end of study and were not evaluated for histopathological findings. Blood was collected on day 21 postpartum from the 250 mg/kg/day group at 0.5, 1, 2, 4, 8, and 24 hours post-dose.
Age: 4 days old at the start of treatment

Weight: Males and females 10 g weight

Doses in administered units: 0, 250, 500, 1000, 2000 mg/kg/day (8/sex/group) were used. Dose selection basis was not provided. Pups were treated from day 4 to day 21 post-partum.

Route, form, volume, and infusion rate: Oral gavage. A constant dose volume of 7.5 ml/kg/day was given to pups for 18 days.

Observations and times:
Clinical parameters, physical development, organ weights, histopathology were determined as shown below.

Administration route: oral (gavage)

Treatment of controls:
1% aqueous carboxymethylcellulose solution
Dose-volume: 7.5 mL/kg/day

Age: 4 days at study start

Body weight at initiation:
males: 10 g females: 10 g

Treatment days per week: 7

Study group	1		2		3		4		5*	
Dosage (mg/kg/day)	0		250		500		1000		2000	
Sex (M/F)	M	F	M	F	M	F	M	F	M	F
Number of test animals										
Principal	8	8	8	8	8	8	8	8	10	11
Satellite	-	-	9	9	-	-	-	-	9	9
Number of animals died or sacrificed in extremis	0	0	4	4	2	2	0	1	19	20

*following the mortality recorded on day 1 of dosing in the animals of group 5, five additional animals (2 males and 3 females) were included in this group from the first day of dosing.

Clinical observations:	yes ☒	no ☐	Clinical Chemistry: yes ☒ no ☐
Food consumption:	yes ☐	no ☒	Urinalysis**: yes ☒ no ☐
Water consumption:	yes ☐	no ☒	Organ weights: yes ☒ no ☐
Body weight:	yes ☒	no ☐	Necropsy: yes ☒ no ☐
Hematology:	yes ☒	no ☐	Histology: yes ☒ no ☐

Additional examinations:
. determination of plasma levels (satellite animals given 250 mg/kg/day) was performed on day 21 at the following times: 0.5 and 4 hours, 1 and 8 hours, 2 and 24 hours after dosing in three different males and females respectively;
. physical and reflex development was assessed on days 5 *post-partum* (pinna unfolding, hair growth and surface righting reflex), 11 *post-partum* (cliff avoidance), 13 *post-partum* (incisor eruption) and 17 *post-partum* (air righting reflex, eye opening and auricular duct opening).

Additional information:
. the satellite animals were killed after blood sampling without laboratory investigations and macroscopic *post-mortem* examination;
. **urinalysis: because of the low number of pups sampled (three) and the low quantity of available urine per pup, only some selected parameters were determined (eg. specific gravity could no be measured).

Urinalysis: This was measured only in the 250 mg/kg/day males on the day of sacrifice.

Gross pathology: At sacrifice in all animals.

Organs weighed: Organs listed in the Table were weighed.

Histopathology: See the list below. Summary histopathology Table did not include all tissues, but these were obtained from appendix data.

Table. Organ weighed and histopathology was performed on following tissues:

Organs	Organ weight	Preservation of tissue	Microscopic examination Groups 1 and 4	Groups 2 and 3
Macroscopic lesions		X	X	X
Adrenals	X	X		
Aorta		X		
Brain (including medulla/pons cerebellar and cerebral cortex)	X	X	X	
Cecum		X		
Colon		X		
Duodenum		X		
Epididymides	X	X	X	X
Esophagus		X		
Eyes with Harderian glands		X		
Femoral bone with articulation		X		
Heart	X	X	X	
Ileum		X		
Jaws (maxillary-mandibular)		X		
Jejunum		X		
Kidneys	X	X	X	
Left femur (bone and cartilage)		X	X	X
Left tibia (bone and cartilage)		X		
Liver	X	X	X	
Lumbar vertebra (L1 to L5) (bone and cartilage)		X		
Lungs with bronchi	X	X	X	
Lymph nodes (mandibular and mesenteric)		X		
Mammary glands/area		X		
Ovaries	X	X	X	X
Pancreas		X		
Pituitary gland		X		
Prostate		X	X	X
Rectum		X		
Right femur (bone and cartilage)		X		
Right tibia (bone and cartilage)		X		
Salivary glands (sublingual and submaxillary)		X		
Sciatic nerve		X		
Seminal vesicles		X	X	X
Skeletal muscle		X		
Skin		X		
Spinal cord (cervical, thoracic and lumbar)		X		
Spleen	X	X		
Sternum with bone marrow		X		
Stomach with forestomach		X		
Testes	X	X	X	X
Thymus	X	X	X	X
Thyroids with parathyroids	X	X		
Tongue		X		
Trachea		X		
Urinary bladder		X		
Uterus (horns and cervix)		X	X	X
Vagina		X	X	X

Results:

Mortality: All doses produced unscheduled deaths, but deaths at lower doses (up to 500 mg/kg/day) were considered gavage accidents (inhaled material) or due to blood drawing. Deaths at high doses (at 2000 mg/kg/day) were considered drug related, and were associated with clinical signs. Most pups in the 2000 mg/kg/day group died within 2-3 days (the last one died on day 14) and had clinical signs of ill health (including coldness to touch, pallor of body extremities, emaciation, dehydration, swollen abdomen, hypokinesia). The incidence of death in males was 0/8, 4/17, 2/8, 0/8, and 19/19 at 0, 250, 500, 1000, and 2000 mg/kg/day, respectively. In females, the incidence of death was 0/8, 4/17, 2/8, 1/8, and 20/20 respectively. No histopathological findings were observed in main study animals at doses up to 1000 mg/kg/day, and no post-mortem

examination was performed in satellite animals. Histopathology was not performed at 2000 mg/kg/day in the main study animals as most died within 2-3 days.

Table. Mortalities in a 2-week toxicity studies in rats.

Dose-level (mg/kg/day)	0	250	500	1000	2000
Male pups	8	17(8+9*)	8	8	19(8+9*+2**)
Found dead	0	0	0	0	19
Found dead . after dosing	0	3	1	0	0
Died after . blood sampling	0	1	1	0	0
Total unscheduled . deaths	0	4	2	0	19
Final sacrifice	8/8	13/17	6/8	8/8	0/19
Female pups	8	17(8+9*)	8	8	20(8+9*+3**)
Found dead	0	2	1	1	20
Found dead . after dosing	0	2	1	0	0
Died after . blood sampling	0	0	0	0	0
Total unscheduled . deaths	0	4	2	1	20
Final sacrifice	8/8	13/17	6/8	7/8	0

*: animals assigned to the satellite groups.
**: supernumerary animals

Some of these mortalities are explained by the sponsor below. Note that 8/34 pups died at 250 mg/kg/day (4/8 of these were accidental deaths, but the other 4 still died at this dose, which were supposedly not accidental**).** At 500 mg/kg/day, all 4/16 pups died accidentally. However the cause of deaths at 1000 and 2000 mg/kg/day are unknown. The total unexplained deaths were 0/16, 4/34, 1/16, 1/16, 39/39 at 0, 250, 500, 1000, and 2000 mg/kg/day respectively (or 0%, 12%, 6%, 6%, and 100% respectively). As indicated above, all 39/39 deaths at 2000 mg/kg/day were considered as drug related by the sponsor, whereas mortality at lower doses was not considered as drug related.

Mortalities
The causes of death in these animals were found to be as follows:

Group	Animal number	Cause of death
2 (250 mg/kg/day)	W24522	Inhaled material, probably the result of an accident at dosing
	W24524	Not evident
	W24581	Emphysema associated with an accident at dosing
	W24585	Emphysema associated with an accident at dosing
3 (500 mg/kg/day)	W24537	Presence of inhaled material in lungs, probably an accident at dosing
	W24542	Not evident
	W24596	Presence of inhaled material in lungs, probably an accident at dosing
	W24601	Abscesses and pericarditis, probably caused by injuring during dosing.

There were no changes that were attributable to the activity of the test compound.

Clinical signs in surviving pups: At 1000 mg/kg/day, 2/8 male pups had clinical signs (one had swollen abdomen from day 13, the other had a wound in a urogenital area from day 8-10 and abnormally colored feces from day 14), however these pups survived through the end of treatment. In female pups, clinical signs were observed at 500 mg/kg/day (one had a wound in a urogenital area on day 6), at 1000 mg/kg/day (one had a wound in a urogenital area on day 13, and one had orange colored feces from day 13), and at 2000 mg/kg/day (one pup had poor clinical signs).

Body weights: Body weight gains were slightly lower in treated vs controls. When compared body weight at the start of carglumic acid treatment, the gain was significantly lower at 1000 mg/kg/day (by 17-20%). The weight gain at 2000 mg/kg/day was 40% lower in males and up to 49% lower in females compared to controls (up to day 11 when all animals has died). These are shown in the Table below.

Table. Body weight gains in a 2-week toxicity studies in rats.

Body weight gain (expressed in grams) of principal and satellite animals over the treatment period is summarized below:

Group mean body weight gain (grams), days 1 to 18 (4 - 21 *post-partum*)

Treatment	Dose-level (mg /kg/day)	Body weight gain (g) 1-4	4-8	8-11	11-18	1-18 (%)	BWC ratio day 18/day 1
Male pups							
Vehicle	0	5.4	10.3	7.8	16.9	40.3	5.8
Test substance	250	5.3	10.2	8.3	18.2	42.0 (+4)	5.4
Test substance	500	6.4	12.3*	10.1#	20.0	48.7** (+21)	5.4
Test substance	1000	4.7	10.0	7.4	16.9	39.1 (-3)	4.9
Test substance	2000	2.0#	4.1#	-	-	- (-)	-
Female pups							
Vehicle	0	5.6	10.3	7.9	16.8	40.6	6.2
Test substance	250	5.3	9.6	8.2	17.5	40.8 (<1)	5.4
Test substance	500	6.0	10.7	10.2*	18.3	45.5 (+12)	5.4
Test substance	1000	4.4	9.0	6.1	16.8	38.3 (-5)	5.1
Test substance	2000	2.1#	5.0#	1.9#	-	- (-)	-

(%): difference from controls expressed in percentage.
-: no surviving animals - BWC: body weight change.
Statistically significant: # p<0.001, * p<0.05, ** p<0.01.

Physical development: No drug-related effects were observed at doses up to 1000 mg/kg/day on reflex or physical development; however, these parameters could not be evaluated at 2000 mg/kg/day beyond day 5, as most pups died in this group.

PUP DEVELOPMENT - number of animals with positive response
SUMMARY TABLE

Sex: male

	0 mg/kg/day		250 mg/kg/day		500 mg/kg/day		1000 mg/kg/day		2000 mg/kg/day	
	N	%	N	%	N	%	N	%	N	%
Physical development										
pinna unfolding on day 5 pp	8	100	17	100	8	100	8	100	17	100
hair growth on day 5 pp	8	100	17	100	8	100	8	100	17	100
incisor eruption on day 13 pp	8	100	17	100	8	100	8	100	1*	100
eye opening on day 17 pp	8	100	17	100	8	100	8	100	0**	-
auricular duct opening on day 17 pp	8	100	17	100	8	100	8	100	0**	-
Reflex development										
surface righting on day 5 pp	8	100	16	94	8	100	8	100	17	100
cliff avoidance on day 11 pp	7	87.5	17	100	8	100	8	100	5*	100
air-righting reflex on day 17 pp	8	100	17	100	8	100	8	100	0**	-

Sex: female

	0 mg/kg/day		250 mg/kg/day		500 mg/kg/day		1000 mg/kg/day		2000 mg/kg/day	
	N	%	N	%	N	%	N	%	N	%
Physical development										
pinna unfolding on day 5 pp	8	100	17	100	8	100	8	100	20	100
hair growth on day 5 pp	8	100	17	100	8	100	8	100	20	100
incisor eruption on day 13 pp	8	100	17	100	7*	100	8	100	3*	100
eye opening on day 17 pp	8	100	15*	100	7*	100	7*	100	0*	-
auricular duct opening on day 17 pp	8	100	15*	100	7*	100	7*	100	0*	-
Reflex development										
surface righting on day 5 pp	8	100	16	94	8	100	8	100	18	90
cliff avoidance on day 11 pp	8	100	17	100	8	100	8	100	5*	83
air-righting reflex on day 17 pp	8	100	15*	100	7*	100	7*	100	0*	-

*: number of surviving animals reduced by mortality
**: no survivors
pp: *post-partum*

Hematology: On day 22, at 500 mg/kg/day in males, WBC (6.8, 7.0, 9.2*, 7.2 G/l at 0, 250, 500, 1000 mg/kg/day, respectively) and leucocytes (5.7, 5.8, 8.1, 5.9 G/l respectively) were increased, RBC were decreased (5, 4.9, 5.4*, 5.2 T/l respectively). In females at 500 mg/kg/day, MCV and MCH were decreased, but these changes were not observed at higher doses.

Clinical chemistry: On day 22, urea levels were low in both sexes at the high dose (males 6.5, 5.5, 5.7, 5* mmol/l respectively, females 6, 5.7, 5.5, 5.1 mol/l respectively). At 1000 mg/kg/day, protein was decreased (48* vs 51 g/l in controls, $p<0.05$) and A/G ratios (2.53* vs 1.86 in controls) were increased in females.

Urinalysis: The results were only provided for males in the 250 mg/kg/day group, due to low volume in the other groups. Urinary pH was low (6.5 vs 7.5 in controls).

Organ weights: At 500 and 1000 mg/kg/day, significant decreases in absolute thymus weight (by 23-30%) and relative weight (by 16-20%) weights were observed. Absolute and/or relative liver weights were increased by up to 25-30% at 500 and 1000 mg/kg/day, but histopathological findings were noted in the liver of only 2/16 pups at 500 mg/kg/day.

Gross pathology: Brownish/blackish/ whitish contents/deposits were observed on the mucosal surface of the stomach in most treated animals, more in females than in males at the high dose. These stomach findings were observed at all doses except at 1000 mg/kg/day (brownish/whitish/yellowish contents or thickened in males with an incidence of 0/8, 3/8, 0/8, 1/8, 2/7 respectively, and in females with an incidence of 0/8, 2/8, 2/8, 0/7, 1-4/8 respectively). In the microscopic exams of stomach, the whitish contents were assumed to be remnants of the drug, the brownish contents seem to be associated with minimal areas of superficial necrosis of mucosa, which the Sponsor considered to be agonal in nature and of no significance. Also other findings were observed in cecum (in males, dilated cecum/yellowish contents in the cecum at 1000 mg/kg/day in 1-3/8 males plus 1/8 females vs none in other groups), kidneys (dilated pelvis in 3/8 vs none in other groups), spleen (enlarged in 1/8 vs none in other groups), thymus (reduced in size or reddish in color in males with an incidence of 0/8, 1/8, 0/8, 1/8 respectively, and in females with an incidence of 1/8 at 500 mg/kg/day), and in the heart (in females at 500 mg/kg/day, heart thickened in 1/8 vs none in other groups). Sponsor states that very few macroscopic abnormalities were detected in the main study animals.

Histopathology: The Sponsor states that no histopathological lesions were observed including in thymus, bone or sexual organs. However some findings were seen in heart (pericarditis or abscess at 500 mg/kg/day in 2/8 females, this may be due to injury during dosing), kidneys (dilated pelvis in 4/8 males at the high dose vs 1/8 in other groups), liver (vacuolated hepatocytes at 500 mg/kg/day in 1/8 M + 1/8 F vs none in other groups), and spleen (extramedullary hematopoiesis in 1/8 males vs 0/8 in other groups). Sponsor states that based on microscopic exams in the main study at 250-1000 mg/kg/day, the cause of death was inhaled material, and not due to carglumic acid toxicity.

Toxicokinetics: Plasma concentration in sexes was not different on day 21 postpartum. Maximal concentrations were noted at 4 hours, and were 39-50 µg/ml at 250 mg/kg/day.

The mean plasma levels of the test substance measured in the 250 mg/kg/day group were as follows:

Time post-dosing (h)	0.5	1	2	4	8	24
Mean plasma level (µg/mL)						
. males	6.74 (3)	11.85 (2)	30.65 (2)	50.4 (3)	13.45 (2)	1.29 (2)
. females	6.60 (1)	9.16 (3)	28.0 (3)	39.1 (1)	11.3 (3)	1.52 (3)

in brackets: number of animals sampled

Oral absorption of the test substance was demonstrated at the dose-level of 250 mg/kg/day. The maximum plasma levels were reached 4 hours after administration. Detectable levels were still observed 24 hours after treatment. No notable gender-difference was observed.

Toxicology summary: In a 2-week oral toxicity study of carglumic acid in newborn rats (4 days old), doses of 0, 250, 500, 1000, and 2000 mg/kg/day were used. Carglumic acid produced similar plasma concentration in males and females at 250 mg/kg/day on day 21 (39-50 µg/ml). Carglumic acid produced deaths at all doses except at 1000 mg/kg/day (0/16, 8/34, 4/16, 1/16, 39/39 at 0, 250, 500, 1000, and 2000 mg/kg/day). At lower doses these were considered gavage or inhaled drug accidents; however, no accidental deaths were noted in the control group, and 4 deaths at 250 mg/kg/day were reported accidental, the other 4 were not explained. At the high dose of 2000 mg/kg/day, most pups died between 2-3 days of dosing. No histopathological or necropsy findings were noted in the main study animals at doses up to 1000 mg/kg/day in 8/sex/group (satellite animals were not subjected to histopathological examination). No histopathology was performed at 2000 mg/kg/day, but deaths at this high dose were considered as drug related. Carglumic acid produced clinical signs in animals prior to death (coldness to touch, pallor of body extremities, emaciation, dehydration, swollen abdomen, hypokinesia), and in surviving animals the incidence of clinical findings were low (swollen abdomen, wound in urogenital area, abnormally colored feces). The orange colored feces at 1000 mg/kg/day may be due to slight modification of the intestinal transit, but it is not being considered as an adverse event. Carglumic acid decreased body weight gains at 1000 and 2000 mg/kg/day (by up to 20% and 49% respectively). Physical or reflex development was unaffected up to 1000 mg/kg/day (some of these tests could not be performed at 2000 mg/kg/day due to early deaths in pups). Carglumic acid decreased BUN at 1000 mg/kg/day (by up to 23 %*, *$p<0.05$), and decreased urinary pH at 250 mg/kg/day (6.5 vs 7.5, this was only measured in low-dose in males). At 500 and 1000 mg/kg/day, thymus weights were decreased (by 16- 23%), and liver weights were increased (by 25-30%). Gross pathology findings were noted in the stomach (brownish/whitish/grayish/ yellowish contents or thickened stomach in 0/16, 5/16, 3/16, 0/15, and 6/15 rat pups, respectively at 0, 250, 500, 1000, and 2000 mg/kg/day, respectively), spleen (enlarged in 1/8 males at 1000 mg/kg/day vs none in other groups), thymus (reduced in size or reddish in color in 1/16, 1/16, and 1/16 pups at 250, 500, and 1000 mg/kg/day respectively). Histopathological findings were observed at 1000 mg/kg/day in kidneys (dilated pelvis in 4/8 males vs 1/8 in other groups), spleen (extramedullary hematopoiesis in 1/8 males vs 0/8 in other groups), and liver (vacuolated hepatocytes at 500 mg/kg/day in 1/8 M + 1/8 F vs none in other groups).

The deaths are a major concern here and were seen at all doses. The deaths were lower at 1000 mg/kg/day (0/16, 8/34, 4/16, 1/16, and 39/39 at 0, 250, 500, 1000, and 2000 mg/kg/day, respectively). Gross pathological findings in the stomach of brownish/ whitish/yellowish contents were also lower at 1000 mg/kg/day (0/16, 5/16, 3/16, 0/15, and 6/15 at 0, 250, 500, 1000, 2000 mg/kg/day, respectively). The lower incidences of abnormal stomach contents at 1000 mg/kg/day correlates with reduced mortality, which suggests that there may be an effect of carglumic acid on GI motility. Note that in distribution studies in rats, a high concentration of radioactivity was noted in the digestive tract and in the small intestine membrane at 3 hours post-dose. The NOAEL or tolerated dose of carglumic acid in a 2-week oral toxicity study in rat pups could not be established due to mortalities even at 250 mg/kg/day (the lowest dose).

Table. Correlation of gross stomach findings with deaths in pups: Note that deaths include the satellite animals, while abnormal stomach findings are only reported for main study animal (no necropsy or histopathology was done for these pups).

Dose (mg/kg/day)	# died (%-mortalities)	# abnormal gross stomach findings (% gross findings)
control	0/16 (0%)	0/16 (0%)
250	8/34 (24%)	5/16 (31%)
500	4/16 (25%)	3/16 (18%)
1000	1/16 (6%)	0/15 (0%)
2000	39/39 (100%)	6/15 (40%)

ADDENDUM: Upon further examination of the data being presented, it is the opinion of this Reviewer that the deaths at 250 to 1000 mg/kg/day carglumic acid were not related to treatment because: 1) there is a lack of dose dependency; there were no significant differences in mortality rate between control and 1000 mg/kg/day. 2) post-mortem gross pathology of the pups was consistent with gavage accidents, i.e. inhaled material in the lungs. In addition, the only significant histopathological findings in the main study animals were the dilated kidney pelvis in male rats at 1000 mg/kg/day (4/8 males vs 1/8 in other groups). Thus, the NOAEL (no observed adverse effect level) is considered to be 500 mg/kg/day, based on the reduced weight gain and the effect in kidneys at 1000 mg/kg/day.

A 26-Week Oral Toxicity Study of Carglumic Acid in Rats ((b) (4) study No. 20330 TCR)

Key study findings: Carglumic acid following 26 weeks of oral administration in rats (0, 500, 1000 mg/kg/day) produced increased salivation at the high dose (0/20, 1/20, and 19/20 rats at 0, 500, and 1000 mg/kg/day respectively). Also the high dose decreased urinary pH in females (5.2* vs 6.4 in controls) and produced toxicity in Harderian gland (necrotizing inflammation in 15/20 vs 6/20 in controls), liver (multicellular hepatic necrosis in 0/20, 1/20, and 3/20 respectively), kidney (interstitial mononuclear cell aggregation 1/20, 5/20, 9/20 respectively), and thymus (lymphoid depletion 1/19, 3/20, 2/20 respectively). Since higher incidences of findings were noted at the high dose, the NOAEL or tolerated dose of carglumic acid in this study is 500 mg/kg/day.

Study no: 20330 TCR
Volume #, and page #: 3.5, page 005

Conducting laboratory and location: (b) (4)

Date of study initiation: 11/2/2000

GLP compliance: yes

QA report: yes (X) no ( )

Drug lot #, and % purity: Several batch numbers were used. These are 05031001P38, 05031001P39, 05031002P42, 08031010P51, and 08031010P52. The first 3 batches have HPA <0.02%, while the last 3 batches have HPA <0.01%.

Formulation/vehicle: 1% aqueous carboxymethylcellulose (CMC). Carglumic acid was grounded and suspended in the vehicle to achieve concentrations of 100 and 200 mg/ml.

Methods (unique aspects): Young rats, 28 days old were used in this study

Dosing:
Species/strain: Rats Crl CD (SD) IGS, BR strain

#/sex/group or time point (main study):10/sex/dose

Satellite groups used for TK analysis and for micronucleus test: 9 males + 9 females were added for TK analysis. A satellite group of 5 M + 5 F were drug treated for 4 weeks and used for micronucleus test, and additional supernumerary 5M +5 F animals were used as positive controls for the micronucleus assay.

Age: 28 days old at the start of treatment

Weight: Males 75-89 g, females 74-93 g.

Doses in administered units: 0, 500, and 1000 mg/kg/day (10/sex/group) were used. Dose selection was based on a previous 2-week range finding study in newborn SD pups (b) (4) study # 20329 TSR) where the no observed adverse effect level was established to be 1000 mg/kg/day, as 2000 mg/kg/day produced deaths in all pups. Based on the above findings, 500 and 1000 mg/kg/day were selected for the current toxicity study. A male fertility study was also a part of the current study. To assess fertility, treatment of males continued through week 28, and mating with additional non-treated females was initiated on week 26. Thus males were treated for 28 weeks and females for 26 weeks. Fertility parameters with seminology including epididymal (motility, count, morphology) and testicular (sperm, weighed and counted) evaluation was performed. In females, fertility was determined by determining the estrous cycles during weeks 12, 13, 18, and 19.

The immunotoxicity evaluation was also part of this toxicity study. The lymphoid organs were weighed, and Peyers's patches in the duodenum, ileum, and/or jejunum were evaluated. Cellularity of the sternal and femoral bone marrow was visually checked.

Route, form, volume, and infusion rate: Oral gavage. 5 ml/kg/day was given once daily for 26 consecutive weeks.

Observations and times:

Clinical signs: once daily

Body weights: weekly

Food consumption: weekly

Teeth exams: In the main study teeth were examined carefully in weeks 1, 2, 3, 4, 8, 13 and 26.

Body length: The body length of surviving animals were examined prior to treatment and in weeks 4, 8, 13 and 25.

Ophthalmoscopy: These were performed in control and high-dose animals in weeks 12 and 25.

Bone mineral density: In the main study, bone mineral density measurement was performed on the first 5/sex/group in weeks 4, 8, 13, 25 and 28 (in males only), and on females were sacrificed in week 27. Bone mineral density was measured by dual energy X-ray absorption (DXA).

Hematology: In weeks 12 and 25.

Bone marrow smears: These were prepared from left femoral bone of all surviving animals at the end of carglumic acid treatment.

Lymphocyte subset: Peripheral blood lymphocyte was isolated on Ficoll-Hypaque density gradient centrifugation.

Clinical chemistry: In weeks 12 and 25.

Urinalysis: In weeks 12 and 25.

Gross pathology: At sacrifice in all animals.

Organs weighed: Organs listed in the Table were weighed.

Histopathology: This was performed at sacrifice in control and high-dose animals.

See the list below. Certain tissues (duodenum, jejunum, ileum, liver, kidneys, thymus, spleen, right femoral bone) were examined at 500 mg/kg/day. Summary histopathology Table did not include all tissues, but these were obtained from appendix data.

Table. Organs weighed and examined in histopathology.

TISSUE PROCEDURE TABLE

Organs	Organ weights	Preservation of tissue	Microscopic examination	PCNA
Macroscopic lesions		X	X	
Adrenals	X	X	X	
Aorta		X		
Brain (including medulla/pons cerebellar and cerebral cortex)		X	X	
Cecum		X	X	
Colon		X	X	
Duodenum		X	X	X
Esophagus		X	X	
Epididymis*	X	X	X	X (right)
Eyes with Harderian glands		X	X	
Right femoral bone with articulation**		X	X	
Heart	X	X	X	
Ileum		X	X	X
Jaws (maxillary-mandibular)		X		
Jejunum		X	X	X
Kidneys	X	X	X	X
Liver	X	X	X	X
Lumbar vertebra (L1 to L5) (bone and cartilage)		X		
Lungs with bronchi		X	X	
Lymph nodes (mandibular and mesenteric)		X	X	
Mammary glands/area		X	X	
Ovaries***	X	X	X	X
Pancreas		X	X	
Pituitary gland		X	X	
Prostate		X	X	
Rectum		X	X	
Right tibia (bone and cartilage)		X		
Salivary glands (sublingual and submaxillary)		X	X	
Sciatic nerve		X	X	
Seminal vesicles		X	X	
Skeletal muscle		X	X	
Skin		X	X	
Spinal cord (cervical, thoracic and lumbar)		X	X	
Spleen	X	X	X	
Sternum with bone marrow		X		
Stomach with forestomach		X	X	
Testis*	X	X	X	X (right)
Thymus	X	X	X	
Thyroids with parathyroids	X	X	X	
Tongue		X		
Trachea		X	X	
Urinary bladder		X	X	
Uterus (horns and cervix)	X	X	X	
Vagina		X	X	

* : right and left organ weighed separately right organ sampled and examined microscopically.

** : for DXA measurements and microscopic examination.

*** : detailed examinations of follicles were not performed as no relevant findings were seen in female genital organs at microscopy.

Fertility parameters: As indicated earlier, a fertility evaluation was also carried out as a part of this study. From week 26, treated males were mated with untreated virgin females. In females, fertility was evaluated by determining the estrous cycles during weeks 12, 13, 18, and 19, and ovaries and uterus were examined for number of corpora lutea, dead or live embryos, early or late resorptions, number and distribution of

implantation sites (uterine scars). In males, seminology including epididymal (motility, count, morphology) and testicular (sperm, weighed and counted) evaluation was performed.

Immunotoxicity: Lymphoid organs were weighed, and Peyers's patchs in the duodenum, ileum, and/or jejunum were observed and cellularity of the sternal and femoral bone marrow was visually checked. At the end of study blood was taken from surviving animals for lymphocyte subset determination.

Cell proliferation was examined by the proliferating cell nuclear antigen (PCNA): Immunoassaying of the ileum, jejunum, duodenum, epididymides, kidneys, liver, testes and ovaries was evaluated in all groups.

Toxicokinetics: Blood samples were collected for TK analysis on day 1 from satellite rats and week 26 from first 8 main study animals in groups 2 and 3 at 0.5, 1, 2, 3, 4, 8, 12, and 24 hours post-dose. Also, 24 hr urine samples were collected to determine carglumic acid levels.

Results:

Mortality: The following deaths occurred: one control male (dead on day 22, no histopathological or necropsy findings) and one male in the 500 mg/kg/day group (dead on day 49, with enlarged liver, but no histopathological findings). Both deaths were considered related to anesthesia. Another male at 500 mg/kg/day was found dead on day 188 (no clinical signs, no histopathological or necropsy findings were noted). Thus none of the mortalities were considered as drug related.

Clinical signs: Pytalism (excess secretion of saliva) was observed in rats (0/10, 1/10, and 10/10 males at 0, 500, and 1000 mg/kg/day respectively, 0/10, 0/10, and 9/10 females, respectively).

Body weights/Food Consumption: Body weights in males at 1000 mg/kg/day in week 26 were slightly decreased (males were 599, 602, 568 g respectively, females were 348, 333, 339 g respectively). Food consumption was lower in the high-dose males in week 26, but were supposedly lower due to the frequent handling of animals due to blood collection for TK, mating, etc (males consumed 24.3, 21.4, and 21.6 g/animal/day respectively). In the high-dose females, food consumption was increased due to spillage (21.5, 21.7, and 27.9 g/animal/day respectively). Body weights are shown in the Table below.

Table. Body weights and weight changes in 26-week toxicity study in rats

Dose-level (mg/kg/day)	0	500	1000
Males			
. body weight, week 1 (g)	82	83	82
. body weight, week 26 (g)	599	602	568
. body weight change (g)	+517	+519	+486
. variation from controls (%)	-	0	-6
Females			
. body weight, week 1 (g)	81	82	81
. body weight, week 26 (g)	348	333	339
. body weight change (g)	+267	+251	+258
. variation from controls (%)	-	-6	-3

The body weight gain of treated males and females was similar to that of controls.

Teeth exams: No drug-related effects were observed.

Body length: No drug-related effects were observed.

Ophthalmoscopy: No drug-related ocular effects were observed.

Bone mineral content or density: These were measured *in vivo* in weeks 4-28 and *ex vivo* at sacrifice. *In vivo* the bone mineral content was determined by dual energy X-ray absorption (DXA) in weeks 4-27/28. Also bone mineral content (BMC in g) and bone mineral density (BMD) were determined *ex vivo* in the right femur and on whole body. Only results on bone mineral density are discussed here. No drug-related effects were observed *ex vivo* on the bone mineral density in the right femur, proximal femoral or distal femoral metaphysis regions in both sexes. The DXA analysis *in vivo* in weeks 4, 8, 13, and 27/28 in males or females also showed no changes in drug treated vs controls.

Hematology: In week 25 in the high-dose males, decreases in APTT (24.2, 23.7, and 19.7* sec respectively) were observed. In females there was a tendency for decreases in APTT levels (16.4, 16.2, and 14.9 sec respectively) and increases in neutrophils (0.71, 1.03*, and 1.05* g/l respectively) at 1000 mg/kg/day..

Clinical chemistry: In week 25 in females, inorganic phosphorus (1.45, 1.37, and 1.21* mmol/l respectively) was decreased, and cholesterol in males was decreased (1.7, 1.2, 1.2 mmol/l respectively).

Urinalysis: Both doses of carglumic acid produced decreases in pH values in both sexes, (see Table below). Also, significant increases in specific gravity were observed in week 12 in the high-dose females (1024, 1035, and 1035* respectively, *$p<0.05$).

Table. Effects of carglumic acid on urinary pH in 26-week toxicity study in rats

Dose-level (mg/kg/day)	0	500	1000
Males			
. pH, week 12	7.4	5.9	6.1
. pH, week 25	7.2	6.0	6.2
Females			
. pH, week 12	6.4	5.9	5.2**
. pH, week 25	6.4	6.0	6.1

**: p<0.01

In absence of histopathological findings in the kidneys, a relationship to treatment with the test substance of the decreased pH values was ruled out.

Organ weights: Absolute and relative kidney, liver, spleen and uterus weights were increased, while thymus weights were slightly decreased at the high dose (see table below).

Table: Effects of carglumic acid on organ weights in a 26-week toxicity study in rats

The following differences (in %) were noted in absolute and relative organ weights between treated and control animals:

	Males		Females	
Dose level (mg/kg/day)	500	1000	500	1000
Kidneys				
. absolute	+10	-0	+5	+9
. relative	+8	+5	+10	+13
Liver				
. absolute	+6	+1	+3	+11
. relative	+5	+7	+9	+15*
Spleen				
. absolute	-9	-5	+5	+12
. relative	-9	+1	+12	+18
Thymus				
. absolute	-16	-19	-16	-9
. relative	-17	-14	-13	-7
Uterus				
. absolute			+1	+18
. relative			+4	+20

* : p<0.05

The higher uterine weight was due to the variation in the phase of estrous cycle (five female rats treated at 1000 mg/kg/day were in the estrous phase of the cycle where the highest uterine weight can be found versus none in the control group).

Gross pathology: In males, liver had grayish/whitish foci (0/10, 1/10, and 2/10 males in the 0, 500, and 1000 mg/kg/day groups, respectively).

Histopathology: Sponsor states that no histopathological lesions were observed, and findings were recognized as commonly occurring changes in rats of this strain and age. The observed lesions occurred with equal incidence and severity in both control and drug treated rats. Sponsor's histopathology summary Table did not include all tissues examined; data for only some tissues were provided. However, the following histopathological findings were observed in the Harderian glands, kidneys, liver, mandibular lymph nodes and thymus at 1000 mg/kg/day. The severity scores were not provided.

Table - Histopathology data with carglumic acid (0, 500, and 1000 mg/kg/day) in the 26-week oral toxicity study in rats:

	(n=10/sex/dose for controls and high dosed rats)	
	Males	Females
Harderian Gland		
Necrotizing inflammation	1/10, 1/2, 6/10	5/10, ne, 9/10
Liver		
Steatosis	0/10, 0/10,1/10	0/10, 0/10, 0/10
Multicell hepato necrosis (minimal)	0/10, 0/10, 3/10	0/10, 1/10, 0/10
Tension lipidosis	1/10, 2/10, 2/10	1/10, 2/10, 1/10
Kidney		
Interstitial mononuclear cell aggregation	0/10, 4/10, 7/10,	1/10, 1/10, 2/10
Lungs		
Cholesterol clefts	0/10, 0/2, 2/10	1/10, ne, 0/10
Mandibular lymph nodes		
Sinusal hemorrhage	0/10, 0/2, 0/10	0/10, 0/1, 1/10
Thymus		
Lymphoid depletion	0/9, 1/10, 2/10	1/10, 2/10, 0/10

ne = Not examined.

Toxicokinetics: The maximal plasma concentration was achieved 3 hours post-dose in males and at 2 hours post-dose in females. No significant sex differences in C_{max} or AUC were noted in week 1 between males and females. AUC (but not C_{max}) values generally increased in a less than dose proportional manner. There was slight accumulation of carglumic acid with time in males, but there was a 3-fold accumulation seen in females at 500 mg/kg/day in week 26 vs week 1 (1009 vs 323 µg•h/ml) (see tables below).

Table . Toxicokinetics of carglumic acid in 26-week oral toxicity study in rats.

Dose	Cmax (µg/ml)		AUC 0-24 hrs (µg.h/ml)	
Mg/kg/day	Males	Females	Males	Females
Week 1				
500	76.7	68.7	477.7	322.7
1000	85.9	83.0	537.3	564.3
Week 26				
500	71.6	95.5	891.1	1009.0
1000	78.0	103.8	716.4	810.4

Other PK parameters are shown in the tables below:

Table. Plasma concentration of the drug over time

Time (h)	500 mg/kg/day		1000 mg/kg/day	
	Male	Female	Male	Female
0.5	20200	18650	12400	33250
1	34600	48700	33000	59850
2	58950	82950	73900	103800
3	71550	58250	78000	93100
4	64600	57800	74850	98650
8	37000	45700	55350	44850
12	6895	5595	13300	14000
24	607	527	1720	1320

Table. Cmax, Tmax, AUC, T1/2, values

Dose-level (mg/kg)	C_{max} (ng/mL)		t_{max} (h)		AUCt (ng.h/mL)		AUC (ng.h/mL)		Extrap. (%)	
	M	F	M	F	M	F	M	F	M	F
500	71550	82950	3	2	534900	562300	537300	564300	0.5	0.36
1000	78000	103800	3	2	708100	804400	716400	810400	1.2	0.80

Dose-level (mg/kg)	Ke (L/h)		$t_{1/2}$ (h)		Cl/F (L/h/kg)		Vd/F (L/kg)	
	M	F	M	F	M	F	M	F
500	0.24	0.26	2.84	2.67	0.93	0.89	3.8	3.4
1000	0.21	0.22	3.36	3.19	1.4	1.2	6.8	5.7

Urine drug concentrations were not significantly different between sexes (males: 131-184 mg/24 hr, females: 75-129 mg/24 hr urine) and the data are shown in the table below:

Table. 24 hr Urine levels of carglumic acid

Dose-level (mg/kg/day)	Mean ± sd (n = 9) amounts of N-carbamyl-L-glutamic acid excreted in 24 hours (mg)	
	Males	Females
500	131 ± 28.4	74.9 ± 15.11
1000	184 ± 38.9	129 ± 36.9

Fertility study in male and rats: As indicated earlier, this fertility study was a sub-part of the 26-week toxicity study in rats. The reproductive parameters and seminology data are shown below.

Reproductive parameters
No effects on estrous cycle or mating parameters were observed. The data in the following table were obtained from the mating of treated males with untreated females.

Table. Mating data in rats

Dose-level (mg/kg/day)	0	500	1000
Paired males + females	9+9	8+8	10+10
Pairs able to mate	9	8	10
Mating index (%)	100	100	100
Pre-coital time (days)	2.7	2.3	3.3
Pregnant females	9	7	9
Fertility index (%)	100	88	90

Male seminology: The motility and morphology of spermatozoids in the epididymides was not affected. However, slightly reduced epididymal and testicular sperm count was noted in males compared to controls at 1000 mg/kg/day, but no associated histopathological findings were noted in male reproductive organs.

Table. Fertility data in males

Dose-level (mg/kg/day)	0	500	1000
Motility of epididymal spermatozoids (%)	49.4*	50.7	62.2
Morphology (% of normal spermatozoids)	95.6	98.5	97.1
Count (10^4/mm^3 of sperm)	47.9	46.4	35.1
Testicular sperm head count (10^6/g of testis)	157.6	138.6	131.8

*: abnormally low values of males W27066, W27067 and W27069 were excluded.

Immunotoxicity: The potential immunotoxicity was examined by determining the weights of lymphoid organs. The thymus weights were lower at the high-dose but were not statistically significant, while spleen weights were slightly higher at the high-dose (see the table below). The bone marrow cellularity in the sternum and femur did not show any abnormalities, and the myeloid/erythroid ratios were not affected. No histopathological findings were observed in the spleen, thymus or lymph nodes. The microscopic exams of Peyer's patches in the intestine showed that carglumic acid did not alter these structures. Lymphocyte subset studies showed that carglumic acid had no effect on T-lymphocyte or B-lymphocyte subpopulations. The quantitative analysis of the proliferating cell nuclear antigen (PCNA) showed large variation within groups and between groups, so it was difficult to see any significant statistical differences. The PCNA results showed no significant changes in the liver, kidneys, testes, intestine and ovaries in drug-treated groups.

The following differences (expressed as %) were noted in absolute and relative spleen and thymus weights between the treated and control groups:

	Males		Females	
Dose-level (mg/kg/day)	500	1000	500	1000
Spleen				
. absolute	-9	-5	+5	+12
. relative	-9	+1	+12	+18
Thymus				
. absolute	-16	-19	-16	-9
. relative	-17	-14	-13	-7

There were no effects in the spleen and the thymus weights which could be attributed to treatment with the test substance, since all the differences observed in the weights of these organs were not dose-related, not statistically significant and/or of opposite trend in the two sexes.

Toxicology summary: In a 26-week oral toxicity study of carglumic acid in young rats (28 days old), doses of 0, 500, and 1000 mg/kg/day were used. There were 3 deaths (1/10 control males on day 22, and 2/10 males at 500 mg/kg/day were found dead on days 49 and 188 respectively), but none were considered to be drug related as no histopathological or necropsy findings were noted. Carglumic acid produced clinical signs (pytalism) mostly at the high dose (0/20, 1/20, and 19/20 at 0, 500, and 1000 mg/kg/day, respectively). It did not produce any effects on teeth, body length, ophthalmoscopy, or on bone mineral density. At 1000 mg/kg/day, the drug produced minor effects on hematological parameters (decreases in APTT in males, 19.7* vs 24.2 sec in controls, $p<0.05$, and increases in neutrophils in females 1.05* vs 0.71 g/l in controls) and clinical chemistry parameters (decreases in inorganic phosphorus, 1.21* vs 1.45 mmol/l in controls). Carglumic acid decreased urinary pH at all doses, which were significant in females at 1000 mg/kg/day (5.2* vs 6.4 in controls). Both doses slightly increased spleen weights (by 5-18% in females) and decreased thymus weights (by 7-19% in both sexes), and the high dose increased liver (by up to 15%) and kidney (by up

to 13%) weights in females. Target organs of toxicity were Harderian gland (necrotizing inflammation in 6/20, 1/2, and 15/20 rats at 0, 500, and 1000 mg/kg/day, respectively), liver (steatosis in 1/10 males, multicellular hepatic necrosis in 0/20, 1/20, and 3/20 rats respectively, tension lipidosis in 2/20, 4/20, and 3/20 rats, respectively), kidney (interstitial mononuclear cell aggregation 1/20, 5/20, and 9/20 rats, respectively), lungs (cholesterol clefts in 2/20 vs 0/20 in controls), mandibular lymph nodes (sinusal hemorrhage in 1/20 vs 0/20 in controls), and thymus (lymphoid depletion in 1/19, 3/20, and 2/20, respectively). The fertility in males or females was not affected. Similarly carglumic acid did not produce immunotoxicity (i.e., no changes in bone marrow cellularity, lymphocyte subset, or Peyer's patches).

ADDENDUM: Upon further examination of the histopathology data, in the opinion of this Reviewer, the frequency of abnormal histopathological findings in lungs, mandibular lymph nodes, and thymus are considered low and at random. Therefore, only the Harderian gland, liver, and kidney are considered target organs of toxicity.

2.6.6.4 Genetic toxicology

Effects of Carglumic Acid on Salmonella/Escherichia Coli Reverse Mutation Test: (AMES TEST)

Key findings: Carglumic acid was negative in the AMES test

Study no: (b) (4) # 800/001

Volume #, and page #: Original submission, Volume 3.8, page 189
Conducting laboratory and location: (b) (4)

Date of study initiation: 7/15/1997

GLP compliance: Yes

QA reports: yes (X) no ( )

Drug lot #, and % purity: Batch #: KLA 1028B/96 b/c K1, 71.1% pure

Formulation/vehicle: Carglumic acid was prepared in DMSO at a concentration of 50 mg/ml

Methods:
Strains/species/cell line: Salmonella typhimurium tester strains TA98, TA100, TA1535, TA1537, TA102

Dose selection criteria:

Basis of dose selection: The dose selection was based on the previous exploratory study in which doses of 52, 164, 512, 1600 and 5000 µg/plate were used in Salmonella strain TA100 in the presence and absence of metabolic activation. Slight toxicity on bacterial lawn were noted at 5000 µg/plate with S9, but no significant decreases in revertants (>than 50% when compared to controls) were observed with increases in doses. In the above assay, no cytotoxicity related to background lawn was noted, and there was no precipitate formation, even at the top dose. Therefore, 5000 µg/plate was the highest dose used in the Salmonella/E. coli strains in the first assay.

Range finding studies: Doses of 52-5000 µg/plate were used in all strains in the presence and absence of metabolic activation, and no cytotoxicity or precipitate was noted at the highest dose.

Test agent stability: Not stated.

Metabolic activation system: Rat liver microsome S9 fraction.

Controls:
Vehicle or negative controls: DMSO

Positive controls:

Chemical	Strains	Concentration (µg/plate)
2 nitrofluorene (2-NF)	TA98	5
t-Butyl hydroperoxide (t-BHP)	TA102	100
9-aminoacridine (9-AA)	TA1537	50
Sodium Azide (NaA)	TA100 and TA1535	10
2-aminoanthracene (2-A)	All the strains with S_9	5

Comments:

Exposure conditions/Study design: The plate incorporation method was used. The tester strains in the plate (in duplicate cultures) were exposed to the vehicle, drug, or positive controls.

Doses used in definitive study: 52, 164, 512, 1600 and 5000 µg/plate

Analysis:
No. of replicates: Triplicate cultures/dose

Counting method: The colonies were counted manually or automatically, using an image analyzer.

Criteria for positive results: If carglumic acid induces an increase in revertant colonies in a dose-dependent manner, and the increase is at least 2 times for strains TA98, TA100, and 3 times for strain TA1535, TA1537 and WP2urvA compared to vehicle controls, carglumic acid would be considered positive.

Summary of individual study findings:

Study validity: Appropriate dose selection was made for this study, and positive control responses were acceptable.

Study outcome: Two experiments were carried out. In the initial dose range study, no toxicity or drug precipitation was observed at doses of 52-5000 µg/plate in the presence or absence of S-9 in any tester strains. In both the first definitive assay (experiment 1 at doses of 52-5000 µg/plate) and a confirmatory assay (experiment 2, at doses of 492-5000 µg/plate), carglumic acid was not mutagenic in any of the tester strains in the presence or absence of metabolic activation. However, a significant increase in the number of revertant colonies was observed with positive controls (with or without S9 mix). The sponsor states that in experiment 1 (without metabolic activation) in TA98 strain at 5000 µg/plate, slight signs of toxicity on bacterial lawn were noted. Similarly at 5000 µg/plate in strain TA1535, TA98, TA1537, TA102 (with metabolic activation), slight signs of toxicity on bacterial lawn were noted. In conclusion, the AMES test was negative.

Summary: Carglumic acid at doses up to 5000 µg/plate was negative in the Ames test in all tester strains.

ADDENDUM: In a separate study entitled "N-Carbamyl-L-Glutamic acid – Bacterial reverse mutation test (Plate incorporation)" (b) (4) study number: 800/010), a different batch of carglumic acid (040307102) also tested negative in the Ames assay at doses up to 5000 µg/plate.

The following tables contain the results from the study:

Strain	Liver S9	Experiment Number	Treatment µg/plate	Mean	Standard Deviation	Dunnett's Signif.
TA-98	+	2	Solvent	33.7	3.06	
			492	30.0	2.65	NS
			878	33.3	2.31	NS
			1568	33.0	6.56	NS
			2800	27.7	5.03	NS
			5000	30.3	2.08	NS
TA-100	+	2	Solvent	113.7	19.50	
			492	88.0	6.24	NS
			878	84.3	12.58	NS
			1568	72.0	20.95	NS
			2800	75.0	19.52	NS
			5000	74.0	22.65	NS
TA-1535	+	2	Solvent	17.3	3.06	
			492	19.7	4.04	NS
			878	19.7	4.16	NS
			1568	18.3	4.51	NS
			2800	16.0	7.00	NS
			5000	14.0	5.00	NS
TA-1537	+	2	Solvent	10.0	1.00	
			492	14.3	4.04	NS
			878	11.3	3.21	NS
			1568	9.3	2.52	NS
			2800	13.0	4.36	NS
			5000	11.0	5.57	NS
TA-102	+	2	Solvent	541.0	46.77	
			492	463.7	79.13	NS
			878	449.3	57.05	NS
			1568	460.0	71.46	NS
			2800	403.3	48.69	NS
			5000	471.3	98.08	NS

Strain	Liver S9	Experiment Number	Treatment µg/plate	Mean	Standard Deviation	Dunnett's Signif.
TA-98	-	2	Solvent	28.3	3.51	
			492	23.0	2.00	NS
			878	18.0	4.36	NS
			1568	21.0	2.00	NS
			2800	20.3	3.21	NS
			5000	15.7	5.77	NS
TA-100	-	2	Solvent	111.7	8.08	
			492	102.7	11.15	NS
			878	101.3	20.98	NS
			1568	100.0	25.71	NS
			2800	76.0	38.30	NS
			5000	66.5	16.26	NS
TA-1535	-	2	Solvent	31.7	4.62	
			492	31.0	4.36	NS
			878	35.3	8.02	NS
			1568	40.3	8.33	NS
			2800	29.3	4.51	NS
			5000	35.3	3.06	NS
TA-1537	-	2	Solvent	8.3	1.15	
			492	9.0	5.20	NS
			878	9.7	3.21	NS
			1568	7.0	2.00	NS
			2800	5.7	3.51	NS
			5000	7.3	3.21	NS
TA-102	-	2	Solvent	376.3	33.47	
			492	290.3	54.24	NS
			878	314.0	63.32	NS
			1568	305.3	49.22	NS
			2800	310.0	64.55	NS
			5000	255.7	60.54	NS

Key to Dunnett's significance: NS - Not significant * $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.005$

Effects of Carglumic Acid on Chromosome Aberrations in Cultured Human Peripheral Blood Lymphocytes (HPBL).

Study no: (b) (4) # 800/009

Volume #, and page #: Original submission, Volume 3.9, page 002

Conducting laboratory and location: (b) (4)

Date of study initiation: 14/1/1998

GLP compliance: Yes

QA reports: yes (X) no ( )

Drug lot #, and % purity: Batch #: KLA 1028B/96 b/cK1, 99.17% pure. This batch supposedly had 0.3% of hydantoin-5-propionic acid (HPA).

Formulation/vehicle: DMSO. Carglumic acid was prepared in DMSO at a concentration of 50 mg/ml. The final concentration of DMSO was 2%.

Methods:
Test strain and Cells: Cultured Human Peripheral blood Lymphocytes (HPBL) was obtained from blood collected from two healthy donors (1M+1F).

Dose selection criteria:
Basis of dose selection: The dose selection was based on the limited solubility of carglumic acid in the vehicle, and toxicity of carglumic acid. Based on solubility, the maximal dose selected was 1408 µg/ml. The mitotic index was used to measure the cytotoxic/cytostatic effect by analyzing 1000 cells from a selection of random fields. The mitotic index (MI) reduction was evaluated and compared to the negative controls. The volume of test article in the culture was 200 µl. The same volume was used for the negative controls (with a final concentration of 2% DMSO). In the first assay (experiment 1), doses selected were 0.5, 1.6, 4.8, 15, 46, 144, 450, and 1408 µg/ml. No precipitate was noted in the culture medium, even at the highest dose. In the second independent assay (Expt. 2), a narrower dose range (24, 44, 78, 140, 248, 442, 788, and 1408 µg/ml) was selected.

Range finding studies: Based on the limited solubility of carglumic acid, the highest dose used in the preliminary assay was 1408 µg/ml.

Test agent stability: Carglumic acid was used within 2 hours. The stability data were not provided.

Metabolic Activation System: Rat liver microsome S9 fraction.

Controls:
Vehicle or negative controls: DMSO

Positive controls: Nitroquinline-N- oxide (NQO, 1.25 µg/ml for non-activated system), Cyclophosphamide (CP, 12.5 µg/ml for activated system).

Exposure conditions/Study design: This assay determines clastogenesis (chromosomal aberrations). Replicate HPBL cells were exposed to various concentrations of carglumic acid. Two assays were performed. In the first assay (Expt.1), the dose range was 0.5-1408 µg/ml, and the treatment time approximately 4 hours. The time of harvest time was 24 hours after initiation of the treatment with or without S9. In the second assay (Expt. 2), the dose range was 24-1408 µg/ml, and the cells were harvested at 24 and 48 hours after initiation of treatment in the absence of S9. In the presence of S9, the treatment

time was short (approximately 4 hours, due to toxicity of S9) and cells were harvested at 48 hours after initiation of the treatment. This was done to assess the possible cell cycle delay which was not examined in the first assay. Positive and negative controls were similarly treated. The scoring of aberrations was performed on 93-100 cells in metaphase (total of 193 to 200 cell in metaphase). At 788 µg/ml (48 hours time point), only 102 cells were examined due to toxicity at this dose.

Doses used in definitive study: In the first assay, 0.5, 1.6, 4.8, 15, 46, 144, 450, and 1408 µg/ml were used. In the second assay 24, 44, 78, 140, 248, 442, 788, and 1408 µg/ml were used.

Analysis:

Counting method: At the end of the study, at least 93-100 metaphase cells/culture were examined for structural aberrations. In the positive controls, 50 metaphase cells/culture were examined.

Criteria for positive results: The results are considered positive if the percentage of cells with aberrations (excluding gaps) are increased in a dose-related manner, a statistically significant increase in relation to the vehicle or negative control is observed, the values in the negative control are in the range of historical solvent control, and the increase in the positive control was statistically significant.

Summary of individual study findings:

Study validity: Appropriate dose selection was made for this study based on toxicity, and positive control responses were acceptable.

Study outcome: In the first assay (Expt.1) no decreases in mitotic index or cytotoxicity were observed. Therefore, concentrations of 144, 450, 1408 µg/ml were selected for scoring for chromosome aberrations. No increases in structural aberrations were noted. However, the sponsor stated that the structural aberrations observed, exclusive of gaps, were mainly chromosome or chromatid deletions.

In the second assay (Expt. 2), at 24 hours without S9, no cytotoxicity was observed as there were no significant decreases in mitotic index (14-32%, Table 1). Doses of 442, 788, 1408 µg/ml were selected for scoring for chromosome aberrations. Carglumic acid from the dose of 442 µg/ml induced a dose-related clastogenic response, which was statistically significant at 788 to 1408 µg/ml (i.e. the increase was 3 times the solvent controls, $p<0.001$, see Table 2 below). Note that the number of cells with aberrations were 23, 30, 43*, and 66** at 0, 442, 788, and 1408 µg/ml respectively.

Table 1. Mitotic index (or cytotoxicity data) in the 2nd assay in HPBL (expt. 2), in the absence of S9 at 24 hours.

CONTINUOUS TREATMENT WITHOUT METABOLIC ACTIVATION

DOSE LEVEL µg/ml		0	24	44	78	140	248	442	788	1408
M.I. %	A	1.8	NE	NE	NE	NE	NE	1.8	1.8	1.5
	B	2.6						2.0	2.6	1.6
	A + B	2.2						1.9	2.2	1.5
M.I. REDUCTION %	A	-	-	-	-	-	-	0	0	17
	B	-	-	-	-	-	-	23	0	38
	A + B	-	-	-	-	-	-	14	0	32

A, B : Replicate cultures
A + B : Both duplicate cultures combined
NE : Not evaluated as no cytotoxicity preventing chromosome aberration analysis was observed at higher dose level(s)

Table 2. Data at 24 hours without S9: Dose related increases in chromosome aberrations were observed in HPBL (expt. 2) in the absence of metabolic activation

24 hour sampling time, - S9

Treatment (µg/ml)	Replicate	Cells scored	Cells with abs. including gaps	Cells with abs. excluding gaps	Significance $	Mitotic Index (mean)
Solvent	A	100	14	14		1.8
	B	100	12	9		2.6
	Totals	200	26	23		(2.2)
442	A	93	21	17		1.8
	B	100	17	13		2.0
	Totals	193	38	30	NS	(1.9)
788	A	100	22	20		1.8
	B	100	27	23		2.6
	Totals	200	49	43	$p \leq 0.01$	(2.2)
1408	A	100	37	34		1.5
	B	100	35	32		1.6
	Totals	200	73	66	$p \leq 0.001$	(1.5)
1.25 NQO	A	25	8	8		
	B	25	9	9		
	Totals	50	17	17	$p \leq 0.001$	

At 48 hours without S9, mitotic index was reduced by 50% at 442 µg/ml and reached 81% at 1408 µg/ml (see Table 3). Therefore, due to presence of cytotoxicity, the metaphase analysis was performed in cultures at 248, 442, 788 µg/ml for scoring. Carglumic acid did not show any significant increases in cells with chromosome aberrations. The sponsor explains that that the extensive cytotoxicity observed at 48 hours may be related to clastogenic response observed at 24 hours of treatment.

Table 3. Mitotic index in HPBL in expt. 2, in the absence of S9 at 48 hours

CONTINUOUS TREATMENT WITHOUT METABOLIC ACTIVATION

DOSE LEVEL µg/ml		0	24	44	78	140	248	442	788	1408
M.I. %	A	2.0	NE	NE	NE	1.3	1.1	0.7	0.4	0.3
	B	1.2				1.6	0.9	0.8	0.5	0.4
	A + B	1.6				1.4	1.0	0.8	0.4	0.3
M.I. REDUCTION %	A	-	-	-	-	35	45	65	80	85
	B	-	-	-	-	0	25	33	58	67
	A + B	-	-	-	-	13	38	56	75	81

At 48 hours with S9, no significant decreases in mitotic index or cytotoxicity were observed (table 4), however a significant increase in cells with chromosome aberrations was observed at one dose (442 µg/ml). The aberrations observed were mainly deletions, but no dose-related trend was noted at 788-1408 µg/ml (Table 5), so this was considered incidental and not biologically relevant.

Table 4. Mitotic index in HPBL in expt. 2, in the presence of S9 at 48 hours

DOSE LEVEL µg/ml		0	24	44	78	140	248	442	788	1408
M.I. %	A	4.3	NE	NE	NE	NE	NE	5.2	4.1	4.1
	B	5.4						5.3	6.1	7.0
	A + B	4.9						5.3	5.1	5.6
M.I. REDUCTION %	A	-	-	-	-	-	-	0	5	5
	B	-	-	-	-	-	-	2	0	0
	A + B	-	-	-	-	-	-	0	0	0

Table 5. Data at 48 hours with S9.
Increased chromosome aberrations in HPBL at one dose, in the 2nd assay (expt. 2) in the presence of metabolic activation.

48 hour sampling time, + S9

Treatment (μg/ml)	Replicate	Cells scored	Cells with abs. including gaps	Cells with abs. excluding gaps	Significance $	Mitotic Index (mean)
Solvent	A	100	3	2		4.3
	B	100	2	1		5.4
	Totals	200	5	3		(4.9)
442	A	100	7	4		5.2
	B	100	5	5		5.3
	Totals	200	12	9	p ≤0.05	(5.3)
788	A	100	3	3		4.1
	B	100	7	2		6.1
	Totals	200	10	5	Ns	(5.1)
1408	A	100	5	2		4.1
	B	100	1	0		7.0
	Totals	200	6	2	Ns	(5.6)
12.5 CP	A	25	10	9		
	B	25	8	6		
	Totals	50	18	15	p ≤0.001	

In both assays, the positive controls (with or without S9 mix) showed a significant increase in aberrations (however data on positive controls were not provided in some assays).

In conclusion, carglumic acid was clastogenic in the confirmatory assay when carglumic acid exposure was longer (24 hours continuous, in Expt 2), in the absence of S9, and with no cytotoxicity. This test was negative when exposure time with drug was shorter (approximately 4 hours, in Expt 1). The test was negative in the presence of S9 (although it was positive at a lower dose but lacked the dose response effect).

Summary: The chromosome aberration test *in vitro* in cultured human peripheral blood lymphocytes was positive for carglumic acid in this assay in the absence of metabolic activation.

Effects of Carglumic Acid on Chromosome Aberrations in Cultured Human Peripheral Blood Lymphocytes (HPBL).

Study no: (b) (4) study # R 980904

Volume #, and page #: Original submission, Volume 3.9, page 257 (ref 17)

Conducting laboratory and location: (b) (4)
(b) (4)

Date of study initiation: 14/10/1998

GLP compliance: Yes

QA reports: yes (X) no ( )

Drug lot #, and % purity: Batch #: 3030804P282. This batch supposedly had 0.1% of hydantoin-5-propionic acid (HPA)

Formulation/vehicle: DMSO. Carglumic acid was prepared in DMSO at a concentration of 200 mg/ml. 0.5% of this was added to culture medium to give a final concentration of 1000 µg/ml.

Results: A second chromosome aberration assay was performed (study # 980904, ref # 17), using a purer batch of carglumic acid (with 0.1% HPA), performing neutralization of the medium, using 7.5% sodium hydrocarbonate. In this assay, the pH values were between 6.74-7.07 (before treatment, without pH adjustment) and 7.13-7.65 (at the end of the treatment, after pH adjustment), as shown in Table 6 below.

Table 6. Second assay in lymphocytes with carglumic acid. Note that the pH corrections were made here in the assay, and carglumic acid tested negative.

A pH neutrality correction was made before treatment using 7.5% sodium hydrocarbonate solution.

	Dose	pH Values				
		Before pH correction	Before treatment	End of treatment		
				S9- (20 h)	S9- (44 h)	S9+
Solvent control	0	7.73	8.01	7.75	7.45	7.72
Test compound	1000	6.74	7.01	7.44	7.28	7.13
	577.4	7.07	7.31	7.51	7.33	7.35
	333.3	7.32	7.63	7.65	7.45	7.44

No significant increase in osmolality (<50 mOsmol/kg) was observed at the maximum concentration tested when compared to the solvent control.

Table 7. Second assay with carglumic acid. Carglumic acid tested negative with and without S9

TEST COMPOUND : CARBAMYL GLUTAMATE (OE 312)

SPONSOR : ORPHAN EUROPE

Assay without S9-mix	CONTINUOUS TREATMENT: 20 HOURS			Assay without S9-mix	CONTINUOUS TREATMENT: 44 HOURS			Assay with S9-mix	4 HOUR TREATMENT SAPMLING TIME: 44 HOURS		
	Sampling time: 20 h after the start of treatment				Sampling time: at the end of treatment				Sampling time: at the end of treatment		
	STRUCTURAL ABERRATIONS				STRUCTURAL ABERRATIONS				STRUCTURAL ABERRATIONS		
DOSES µg/ml	Break per cell	Abnormal cells excluding gaps only n=200	Abnormal cells including gaps only n=200	DOSES µg/ml	Break per cell	Abnormal cells excluding gaps only n=200	Abnormal cells including gaps only n=200	DOSES µg/ml	Break per cell	Abnormal cells excluding gaps only n=200	Abnormal cells including gaps only n=200
Controls 0	0.025 ±0.157	5	7	Controls 0	0.015 ±0.122	3	6	Controls 0	0.01 ±0.1	2	3
333.3	0.03 ±0.171 N.S.	6 N.S.	9 N.S.	333.3	0.01 ±0.1 N.S.	2 N.S.	10 N.S.	333.3	0.015 ±0.122 N.S.	3 N.S.	8 N.S.
577.4	0.02 ±0.14 N.S.	4 N.S.	6 N.S.	577.4	0.015 ±0.122 N.S.	3 N.S.	8 N.S.	577.4	0.015 ±0.122 N.S.	3 N.S.	7 N.S.
1000	0.01 ±0.1 N.S.	2 N.S.	5 N.S.	1000	0.02 ±0.14 N.S.	4 N.S.	8 N.S.	1000	0.015 ±0.122 N.S.	3 N.S.	4 N.S.

N.S. : not significant on the threshold of p=0.05
n= number of cells scored per dose
Student's t-test for break per cell
χ^2 test for damaged cells with and without gaps

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In conclusion, carglumic acid was not clastogenic in this repeat assay in the presence or absence of S9, using purified drug (with 0.1% HPA), lower DMSO (0.5%), and adjustment of pH (to 7.13-7.65). Carglumic acid exposure in the assay was longer in the absence of S9 (continuous for 20-44 hours, with significant toxicity), and it was shorter with S9 (4 hours Treatment + 44 hours harvest) with no cytotoxicity.

In the previous assay, carglumic acid was positive, because it was supposedly less pure (with 0.3% HPA). Also,a higher DMSO concentration (2%) was used, and the pH of the medium was not adjusted and was probably lower as a result.

Addendum: It is noted that no positive control compound was tested in this study.

Effects of Hydantoin-5-Propionic acid (HPA) in AMES assay

Study no: (b) (4) study # R 980804

Volume #, and page #: Original submission, Volume 3.9, page 215 (reference 16).

Conducting laboratory and location: (b) (4)
(b) (4)

Date of study initiation: 7/29/1998

GLP compliance: Yes

QA reports: yes (X) no ( )

Drug lot #, and % purity: HPA Batch #: 03149806R21

Formulation/vehicle: DMSO.

Results: An AMES assay was conducted to look at the mutagenic potential of the HPA impurity in carglumic acid. This assay was performed in *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537, and *E. coli* strains WP2pKM101 and WP2uvrA.pk101. HPA was not mutagenic in any tester strains at doses up to 5000 µg/plate in the presence or absence of metabolic activation. Thus, HPA was negative in AMES assay.

- STRAINS USED : S. typhimurium: TA 1535, TA 1537, TA 98, TA 100
  E. coli: WP2pKM101 and WP2uvrA.pKM101
- PRELIMINARY TOXICITY TEST : Carried out on 6 strains - Incubation period : 48 hours
- STERILITY TEST : Normal
- MUTAGENICITY TEST : Carried out both with and without metabolic activation using hepatic microsomes from rat livers induced by Aroclor 1254
  Incubation period : 48 hours
- NUMBER OF ASSAYS : 2 (the second assay with metabolic activation was performed using pre-incubation protocol)
- INITIAL SOLUTION : DMSO
- LIMITING FACTOR FOR MAXIMUM DOSE : highest dose recommanded by OECD
- DOSES USED IN MAIN TEST :

WITHOUT S9-mix

Strain	TA 1535		TA 1537		TA 98		TA 100		WP2pKM101		WP2uvrApKM101	
ASSAY	1	2	1	2	1	2	1	2	1	2	1	2
DOSES µg/plate	0	0	0	0	0	0	0	0	0	0	0	0
	50	50	50	50	50	50	50	50	50	50	50	50
	150	150	150	150	150	150	150	150	150	150	150	150
	500	500	500	500	500	500	500	500	500	500	500	500
	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500
	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000

WITH S9-mix

Strain	TA 1535		TA 1537		TA 98		TA 100		WP2pKM101		WP2uvrApKM101	
ASSAY	1	2 *	1	2 *	1	2 *	1	2 *	1	2 *	1	2 *
DOSES µg/plate	0	0	0	0	0	0	0	0	0	0	0	0
	50	50	50	50	50	50	50	50	50	50	50	50
	150	150	150	150	150	150	150	150	150	150	150	150
	500	500	500	500	500	500	500	500	500	500	500	500
	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500
	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000

* Assay with pre-incubation

RESULTS :

The compound Hydantoïne 5-propionic acid provided by ORPHAN Europe induced no mutagenic activity in the four Salmonella typhimurium and the two Escherichia coli strains tested.

Conclusion: HPA at doses up to 5000 µg/plate was negative in the Ames test in all tester strains.

Addendum: It is noted that no positive control compound was tested in this study.

Sponsor states that the impurity HPA alone demonstrated a clastogenic effect (the data on this assay are not provided) at a concentration which did not induce pH variations at the highest dose of 2500 µg/ml without metabolic activation. However they state that this did not explain the positive effect observed at low concentration of HPA (0.3%). Therefore, the next chromosome aberration assay was carried out with neutralization of the medium (pH values were 7.18-7.94), to see if pH variations play a role in the positive response of carglumic acid in this assay.

Effects of Carglumic Acid on Chromosome Aberrations in Cultured Human Peripheral Blood Lymphocytes (HPBL) with pH Adjustments

Study no: (b) (4) study # R 981108

Volume #, and page #: Original submission, Volume 3.9, page 299 (reference #18)

Conducting laboratory and location: (b) (4) (b) (4)

Date of study initiation: 23/10/1998

GLP compliance: Yes

QA reports: yes (X) no ( )

Drug lot #, and % purity: Batch #: 3030804P282. This batch supposedly had 0.1% of hydantoin-5-propionic acid (HPA)

Formulation/vehicle: DMSO. Carglumic acid was prepared in DMSO at a concentration of 200 mg/ml. 0.5% of this was added to culture medium to give a final concentration of 1000 µg/ml.

Results: This chromosomal aberration assay was conducted to check the variations in pH with carglumic acid. The assay was performed only in the absence of S9. Carglumic acid was dissolved in DMSO at 200 mg/ml, but was added at 0.5% to the culture medium. The treatment time was 24 hours. In this study, the pH values before treatment were between 6.51-7.32, and at end of treatment were between 7.18-7.94 (see Table 8).

Table 8. Carglumic acid was subjected to neutralization to make pH corrections. The pH values are provided before and after drug treatment in the assay.

Doses µg/ml	WITHOUT NEUTRALIZATION pH VALUES			WITH NEUTRALIZATION pH VALUES		
	Before treatment	At the end of treatment		Before treatment	At the end of treatment	
		Culture 1	Culture 2		Culture 1	Culture 2
Solvent Control	7.32	7.35	7.39	-	-	-
1000	6.51	6.95	7.00	7.18	7.55	7.60
560.2	6.83	7.2	7.26	7.57	7.65	7.65
313.9	7.03	7.29	7.33	7.94	7.67	7.71

The highest dose of 1000 µg/ml carglumic acid produced toxicity when not neutralized (mitotic index was 39% compared to solvent control). Toxicity was lower (71% mitotic index compared to control) when carglumic acid was neutralized.

Table 9. Cytotoxicity of carglumic acid with pH corrections.

4 Hour Treatment	DOSE µg/ml	SLIDE	MITOTIC INDEX (*) PER SLIDE	MITOTIC INDEX (*) MEAN	% OF CONTROL	χ^2	p
SOLVENT CONTROLS	0	975 983	12.6 5.9	9.3			
TEST COMPOUND WITHOUT NEUTRALIZATION	1000	977 985	5.1 2.1	3.6	**38.7**	53.096	<0.001
	560.2	978 986	9.8 6	7.9	**84.9**	2.325	N.S.
	313.9	979 987	9.7 5.7	7.7	**82.8**	3.097	N.S.
TEST COMPOUND WITH NEUTRALIZATION	1000	980 988	7.2 6	6.6	**71**	9.624	<0.01
	560.2	981 989	12.4 5.3	8.9	**95.7**	0.194	N.S.
	313.9	982 990	11.3 5.1	8.2	**88.2**	1.384	N.S.

(*) expressed as percent of cells undergoing mitosis and based on 1000 cells counted
N.S.: not statistically significant on the threshold of p=0.05

Carglumic acid tested negative when pH was adjusted, but was positive at 560-1000 µg/ml when pH was not adjusted. Thus this study demonstrated that slight changes in pH can alter the response to carglumic acid.

Table 10. Third assay in lymphocytes with carglumic acid. Under neutral pH (7.2-7.9) carglumic acid tested negative, but as lower pH (7-7.4) carglumic acid tested positive.

ASSAY 1	24-HOUR TREATMENT WITHOUT NEUTRALIZATION			ASSAY 1	24-HOUR TREATMENT WITH NEUTRALIZATION		
	Sampling time: at the end of treatment				Sampling time: at the end of treatment		
	STRUCTURAL ABERRATIONS				STRUCTURAL ABERRATIONS		
DOSES µg/ml	Break per cell	Abnormal cells excluding gaps only n=200	Abnormal cells including gaps only n=200	DOSES µg/ml	Break per cell	Abnormal cells excluding gaps only n=200	Abnormal cells including gaps only n=200
Controls 0	0.02 ±0.14	4	7	Controls 0	0.02 ±0.14	4	7
313.9	0.02 ±0.14 N.S.	4 N.S.	10 N.S.	313.9	0.035 ±0.184 N.S.	7 N.S.	8 N.S.
560.2	0.07 ±0.256 p<0.05	14 p<0.05	20 p<0.01	560.2	0.035 ±0.184 N.S.	7 N.S.	10 N.S.
1000	0.07 ±0.256 p<0.05	14 p<0.05	20 p<0.01	1000	0.04 ±0.221 N.S.	7 N.S.	10 N.S.

n= number of cells scored per dose
Student's t-test for break per cell
$\chi 2$ test for damaged cells with and without gaps
N.S.: not statistically significant on the threshold of p=0.05

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In conclusion, without pH neutralization (at the end of treatment pH values were 6.95-7.39), carglumic acid was clastogenic, but with neutralization of the medium (pH values were 7.18-7.94), no clastogenic activity was observed. These studies suggest that carglumic acid is negative if the pH of the assay is neutral. The content (%) of HPA may or may not be important. However, HPA is also clastogenic in the lymphocyte aberration assay at high doses (2500 µg/ml). It is unclear if carglumic acid with impurity of 0.3% HPA would increase the clastogenic potential.

Effects of Carglumic Acid on *In Vivo* Micronuclei in Rats

Key findings: Carglumic acid was positive in the *in vivo* micronuclei assay.

Study no (b) # 800/004

Volume #, and page #: Original submission, Volume 3.8, page 290 (reference 11)

Conducting laboratory and location: (b) (4).

Date of study initiation: 10/13/1997

GLP compliance: Yes

QA reports: yes (X) no ( )

Drug lot #, and % purity: Batch #: KLA 1028B/96 b/c K1, 99.11% pure. This contained

0.3% HPA (impurity)

Formulation/vehicle: 1% CMC in water. Carglumic acid suspension was prepared to achieve 10 and 20 ml/kg volume. In a pilot study rats received 10 ml/kg, in the main study it was 20 ml/kg, since the suspension was too viscous in a pilot study.

Methods:

Test strain and Cells: Sprague Dawley (ICo, OFA.SD) rats, males and females 5-6 weeks of age.

Dose selection criteria:
Basis of dose selection: The dose selection was based on a pilot study in rats (in 3M +3F per group), where rats were given oral (gavage) doses of 690-7040 mg/kg/day. Rats were observed for clinical signs/mortality and body weights for 48 hours post dosing. The drug did not produce any mortalities or clinical signs, and no marked decreases in PCE/NCE were observed at three high doses. Therefore in the main micronucleus test one high dose of 7040 mg/kg/day was used. In the main test, no mortality was noted but clinical signs (diarrhea in both sexes in 9/10 animals) were observed.

Range finding studies: Oral doses of 690-7040 mg/kg/day were used in the range finding study.

Test agent stability: This was freshly prepared, stability unknown.

Controls:
Vehicle or negative controls: Sterile distilled water

Positive controls: Cyclophosphamide (CP), 60 mg/kg/day by IP route.

Exposure conditions/Study design: This assay determines clastogenesis, or the chromosome damaging activity *in vivo*. Erythroblasts in the bone marrow undergoing their last chromosome replication are the target cells here. In the main study, rats (5/sex/group/sacrifice time) were given a single oral dose of carglumic acid at 7040 mg/kg/day. A group of rats was similarly treated with the vehicle (1% CMC) or positive control (CP). Animals were sacrificed at 24 hours and 48 hours, and bone marrow cells were prepared. At 7040 mg/kg/day in the main study on day 2, diarrhea was observed in both sexes in a total of 9/10 rats.

Doses used in definitive study: oral doses of 7040 mg/kg were used.

Analysis:
No. of animals used: 5/sex/group/sacrifice time

Counting method: Polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) were analyzed, and examined for the presence of micronuclei.

Criteria for positive results: If a dose response increase in micronucleated PCE (MPCE) is observed, and if one or more doses compared to the vehicle control (in mean MPCE) are significantly increased, and if the incidence of MPCE for the positive control is statistically significant, carglumic acid would be considered positive.

Summary of individual study findings:

Study validity: Higher doses could have been used to determine the maximal tolerated dose. However this dose is still acceptable, and positive control responses were acceptable.

Study outcome: Carglumic acid induced a decrease in PCE/NCE ratio after 24 hours. A statistically significant increase in mean incidence of micronucleated PCE was noted in both sexes combined. This increase was not sex-dependent and greatest (4.72 vs 0.84, $p< 0.05$, approximately 6 times mean the negative control) at 48 hours after dosing at 7040 mg/kg. Cyclophosphamide (CP-529414, the positive control) induced a significant increase in the MPCE in both male/female rats, compared to vehicle control (14.5 vs 1.24 in controls, $p<0.05$). Re-examination of the effect of carglumic acid in the pilot study showed that carglumic acid was positive at two high doses of 3940 and 7040 mg/kg/day (3.61-3.87 vs 0.84 in controls, see Table 13). However at a lower dose (2210 mg/kg or below) the response was within a normal range (1.73 vs 0.84). In conclusion, carglumic acid was clastogenic in this assay.

Table 11. **PCE/NCE ratios in a preliminary study at 48 hours, and PCE/NCE ratios in the main study at 24 hours**

Individual data of PCE/NCE ratio in the preliminary study, 48 hours after dosing

Dose (mg/kg)	Sex	Ratio PCE/NCE	Mean (± S.D.)
2210	Males	0,79 0,74 0,80	0,67 (± 0.13)
	Females	0,52 0,67 0,52	
3940	Males	0,52 0,76 0,75	0,63 (± 0.16)
	Females	0,47 0,81 0,46	
7040	Males	0,67 0,69 0,51	0,51 (± 0.15)
	Females	0,45 0,31 0,45	

Individual data and statistical analysis of PCE/NCE ratio in the main study - Sampling time 24 hours

Group description	Dose	Group number	Ratio PCE/NCE	Mean (+ S.D.)	t-value	Significance
Negative control	0 (mg/kg)	1 Males	0,53 1,43 0,46 1,17 0,31	0,68 (± 0.36)	NA	NA
		1 Females	0,51 0,45 0,79 0,51 0,61			
Positive control	60 (mg/kg)	3 Males	0,24 0,35 0,18 0,25 0,15	0,24 (± 0.08)	-3,773	*
		3 Females	0,29 0,37 0,16 0,23 0,19			
Test article	7040 (mg/kg)	4 Males	0,4 0,58 0,56 0,36 0,45	0,4 (± 0.11)	-2,352	*
		4 Females	0,4 0,39 0,21 0,34 0,34			

Sampling time 48 hours

Negative control	0 (mg/kg)	2 Males	0,49 0,67 1,38 1,9 0,85	0,86 (± 0.44)	NA	NA
		2 Females	0,72 0,68 0,75 0,6 0,58			
Test article	7040 (mg/kg)	5 Males	0,61 0,56 1,24 0,74 0,85	0,66 (± 0.24)	-1,262	NS
		5 Females	0,66 0,61 0,35 0,5 0,51			

PCE: polychromatic erythrocytes; NCE: normochromatic erythrocyte; S.D.: standard deviation
NS: no significant decrease; NA: not applicable; * : significant decrease ($p \leq 0.05$)

Table 12. Increase in MPCE in the main study at 24 hours and 48 hours.

Sampling time 24 hours

Group description	Dose	Group number		MPCE ‰	By Sex: Mean (± S.D.)	By Sex: t-value	By Sex: Significance	Sexes combined: Mean (± S.D.)	Sexes combined: t-value	Sexes combined: Significance
Negative control	0 (mg/kg)	1	Males	0,50 0,49 1,98 1,46 0,00	0,89 (± 0.8089)	NA	NA	1,24 (± 1.0751)	NA	NA
			Females	2,96 2,00 2,48 0,50 0,00	1,59 (± 1.2800)	NA	NA			
Positive control	60 (mg/kg)	3	Males	16,95 24,80 19,86 20,87 19,82	20,46 (± 2.8326)	14.855	*	14,55 (± 6.6572)	6,242	*
			Females	8,00 5,45 8,91 10,96 9,89	8,64 (± 2.0978)	6,415	*			
Test article	7040 (mg/kg)	4	Males	5,46 0,99 1,49 4,97 2,49	3,08 (± 2.0298)	2,241	NS	2,77 (± 1.8093)	2,299	*
			Females	3,94 4,42 0,49 2,49 1,00	2,47 (± 1.7353)	0,913	NS			

Sampling time 48 hours

Group description	Dose	Group number		MPCE ‰	By Sex: Mean (± S.D.)	By Sex: t-value	By Sex: Significance	Sexes combined: Mean (± S.D.)	Sexes combined: t-value	Sexes combined: Significance
Negative control	0 (mg/kg	2	Males	0,50 0,98 2,45 0,00 1,49	1,08 (± 0.9431)	NA	NA	0,84 (± 0.8047)	NA	NA
			Females	0,00 0,00 0,99 1,49 0,50	0,60 (± 0.6469)	NA	NA			
Test article	7040 (mg/kg	5	Males	3,44 2,47 2,97 4,45 12,29	5,12 (± 4.0720)	2,161	NS	4,72 (± 2.9083)	4,066	*
			Females	6,68 4,47 3,48 3,98 2,99	4,32 (± 1.4302)	5,299	*			

MPCE: micronucleated polychromatic erythrocytes; S.D.: standard deviation
NS: no significant decrease; NA: not applicable; * : significant decrease ($p<0.05$)

Table 13. Increase in MPCE in the preliminary study at 48 hours.

Sampling time 48 hours (Sexes combined)

					(1) Against Negative control		Against concurrent low dose level	
Group description	Dose	Sex	MPCE ‰	Mean (± S.D.)	t-value	Significance	t-value	Significance
(1) Negative control	0 (mg/kg)	Males	0,50 0,98 2,45 0,00 1,49	0,84 (± 0.8047)	NA	NA	-	-
		Females	0,00 0,00 0,99 1,48 0,50					
Test article	690 (mg/kg)	Males	1,00 1,99 2,00	1,49 (± 0.7747)	1,601	NS	NA	NA
		Females	0,97 0,50 2,49					
Test article	1240 (mg/kg)	Males	0,99 1,46 1,49	1,89 (± 1.0579)	2,095	NS	0,747	NS
		Females	1,95 1,50 3,96					
Test article	2210 (mg/kg)	Males	2,94 1,49 0,98	1,73 (± 1.0239)	1,819	NS	0,458	NS
		Females	1,49 0,50 2,99					
Test article	3940 (mg/kg)	Males	2,38 1,49 0,50	3,87 (± 2.8776)	2,521	*	1,956	NS
		Females	5,97 7,94 4,96					
Test article	7040 (mg/kg)	Males	4,48 2,49 2,98	3,61 (± 1.4976)	4,183	*	3,080	*
		Females	6,27 2,45 2,99					

(1) : Negative control of the main study, 48-hour sampling time.

Summary: Carglumic acid was clastogenic at doses of 3940-7040 mg/kg in an *in vivo* micronucleus test in rats. A reproducible positive effect was observed at 7040 mg/kg/day at 24 and 48 hours.

Conclusion: Carglumic acid was positive in the rat micronucleus assay.

Effects of Carglumic Acid on *In Vivo* Micronucleus Test in the Rat

Note: This study compared two batches of carglumic acid containing different levels of the HPA impurity.

Study no: (b) (4) study # 990505

Volume #, and page #: Original submission, Volume 3.9, page 331 (reference 19)

Conducting laboratory and location: (b) (4) (b) (4)

Date of study initiation: 17/3/1999

GLP compliance: Yes

QA reports: yes (X) no ( )

Drug lot #, and % purity: Batch #: 03030804P282 (contained 0.1% hydantoin-5-propionic acid (HPA).

Batch #: KLA 1028B (containing 0.3% HPA)

Formulation/vehicle: 0.5% CMC, 20 ml/kg

Methods:
Test strain and Cells: Sprague Dawley rats (b) (4), (b) (4), males and females 5/sex/dose, age 5-6 weeks old, weighing 200 g.

Basis of dose selection: The authors stated that the highest dose tested was the maximum tolerated dose, but no basis for this claim was provided.

Range finding studies: Doses were selected based on a previous study.

Test agent stability: This was freshly prepared, stability unknown.

Controls:
Vehicle or negative controls: 0.5% CMC

Positive controls: Cyclophosphamide (CP), 25 mg/kg by IP route.

Exposure conditions/Study design: In the main study, rats (5/sex/group/sacrifice time) were given a single oral dose of carglumic acid at 2000 and 7000 mg/kg. A group of rats was similarly treated with the vehicle (0.5% CMC) or positive control (CP). Animals were sacrificed at 24 hours and 48 hours, and bone marrow cells were prepared. No clinical signs, mortality or changes in bodyweight were observed.

Doses used in definitive study: oral doses of 2000 and 7000 mg/kg were used.

Analysis:
No. of animals used: 5-7/sex/group/sacrifice time

Counting method: Polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) were analyzed, and examined for the presence of micronuclei.

Criteria for positive results: If a dose-related increase in micronucleated PCE (MN-PCE) is observed, and if one or more doses compared to the vehicle control (in mean MN-PCE) are significantly increased, and if the incidence of MN-PCE for the positive control is statistically significant, carglumic acid would be considered positive.

Summary of individual study findings:
Study validity: Dose selection was acceptable and positive control responses were also acceptable.

Study outcome: This second assay was performed (study # 990505), using a purer batch of carglumic acid (with 0.1% HPA), and also using an older batch of carglumic acid which contained higher HPA content (i.e. 0.3%). Two doses of carglumic acid (2000 and 7000 mg/kg/day) were used, and 2000 PCEs were scored. The Sponsor stated that carglumic acid tested negative with both old and new batches of carglumic acid (see Table 14 below).

Table 14. Second *in vivo* micronucleus test in rats with old (0.3% HPA) and new (0.1% HPA) batches of carglumic acid.

Species/strain	: Sprague Dawley rats; supplier : (b) (4)
Number of animals	: 70 (5 males and 5 females per group)
Target cells	: *erythroblasts*
Test for induction of	: micronuclei
Treatment of the negative control	: vehicle: 0.5% carboxymethylcellulose , oral route, 20 ml/kg
Test compound	: N-CARBAMYL GLUTAMIC ACID , Batchs # KLA1028B and # 03030804P282
Doses	: 7000 and 2000 mg/kg for 24 h sampling time 7000 and 2000 mg/kg for 48 h sampling time
Administration route	: oral
Treatment schedule	: 1 treatment at T0
Sampling times	: 5 males and 5 females/dose at T0+ 24 hours and at T0 + 48 hours
Positive control	: cyclophosphamide
Dosage	: 25 mg/kg
Administration route	: intraperitoneal
Treatment schedule	: one treatment at T0
Sampling Time	: 5 males and 5 females at T0 + 24 hours
Number and sex of animals analysed per group	: 5 males and 5 females per time of sacrifice
Number of cells analysed per animal	: 2000 PCE

Toxic/cytotoxic effects : the highest dose tested of the compound given by the oral route was 7000 mg/kg , this dose presented a cytotoxic activity in rat bone marrow.

	Polychromatic/normochromatic erythrocyte ratio (M+ F)							mean±sd
GROUP	24 hours			p	48 hours			p
Negative control	**2.58**	±	0.48		**2.98**	±	0.55	
Positive control	**1.12**	±	0.3	<0.001				
7000mg/kg Batch # KLA1028B	**2.06**	±	0.47	<0.05	**2.49**	±	0.59	N.S.
2000mg/kg Batch # KLA1028B	**2.79**	±	0.72	N.S.	**2.87**	±	0.55	N.S.
7000mg/kg Batch # 03030804P28	**2.01**	±	0.59	<0.05	**2.65**	±	1	N.S.
2000mg/kg Batch # 03030804P28	**2.19**	±	0.51	N.S.	**3.6**	±	1.23	N.S.

Genotoxic effects : **The test compound induced no genotoxic activity by the oral route in rat bone marrow**

	Micronucleated polychromatic erythrocyte for 1000 PCE (M+F)							mean±sd
GROUP	24 hours			p	48 hours			p
Negative control	**0.35**	±	0.34		**0.35**	±	0.47	
Positive control	**9.75**	±	2.15	<0.01				
7000mg/kg Batch # KLA1028B	**0.75**	±	0.49	N.S.	**0.45**	±	0.37	N.S.
2000mg/kg Batch # KLA1028B	**0.2**	±	0.35	N.S.	**0.3**		0.42	N.S.
7000mg/kg Batch # 03030804P28	**0.6**	±	0.32	N.S.	**0.55**		0.55	N.S.
2000mg/kg Batch # 03030804P28	**0.6**	±	0.7	N.S.	**0.55**		0.55	N.S.

Effects of the positive control : the positive control, cyclophosphamide, induced at the dose of 25 mg/kg by the I.P. route a significant genotoxic activity in rat bone marrow.

Statistical analysis:

Mann Whitney U rank test for micronuclei

Student't test for PCE/NCE ratio

Table 14 shows that carglumic acid produced significant cytotoxicity at 24 hours with both batches of carglumic acid at 7000 mg/kg. However, it did not induce a significant increase in PCEs at 24 or 48 hours at any dose using either the old and new batch of carglumic acid (0.3% and 0.1% HPA, respectively). Cyclophosphamide (CP, the positive control) induced a significant increase in the mean PCE (9.75 vs 0.35 in controls, $p<0.01$). In conclusion, carglumic acid at doses up to 7000 mg/kg was not clastogenic in the rat bone marrow. No explanation was provided as to why the micronucleus assay was previously positive in the preliminary and confirmatory assay (see previous study), but was negative even when the identical older batch of carglumic acid was tested in the current study.

Effects of Carglumic Acid on *In Vivo* Micronucleus Test in the Rat After 4 Weeks of Treatment with Carglumic Acid at 500 and 1000 mg/kg/day

Study no (b) (4) study # 20334 MAR. This study was performed as a part of the 26-week toxicity study in rats (b) (4) study # 20330 TCR)

Volume #, and page #: Original submission, Volume 3.9, page 80.

Conducting laboratory and location: (b) (4)

Date of study initiation: 11/2/2000

GLP compliance: Yes

QA reports: yes (X) no ( )

Drug lot #, and % purity: 05031001P38, 05031001P39, 05031002P42; >99%; <0.02% hydantoin-5-propionic acid

Formulation/vehicle: 1% CMC

Results: In this study, 5 rats/sex/dose were given the vehicle, 500, and 1000 mg/kg/day of carglumic acid (5 ml/kg/day) orally by gavage for 4 weeks. Also, a positive control group received cyclophosphamide (15 mg/kg/day). Animals were sacrificed on day 29, and bone marrow cells were prepared at 24 hours and 48 hours post-dose. MPE were counted in 2000 polychromatic erythrocytes, and PE:NE ratio was established by scoring total of 1000 erythrocytes (PE+NE). No mortality was noted in this study, and no clinical signs were observed.

A significant effect was noted in males at 1000 mg/kg/day, but the sponsor concludes that no dose relationship is noted, so the test is negative (see Tables 15-17 below).

Table 15

Test cells :	Rat bone marrow erythrocytes
Test for induction of :	Chromosomal aberrations (Micronuclei)
Species / breed :	Sprague-Dawley rat, Crl CD® IGS BR, COBS-VAF® / (b) (4)
Number of animals :	30 (15 males & 15 females) and 10 supernumerary animals used as positive controls
Formulation of test substance:	carglumic acid (**supplier:** (b) (4) batches No 05031001/P38, P39 and P42) in 1 % carboxymethylcellulose in purified water.
And final concentration :	500 - 1000 mg/kg/day
(in a previous 2-week study - (b) *study 20329 TSR*- performed on newborn SD pups, the NOEL was 500 mg/kg/day, the NOAEL was 1000 mg/kg/day and the dose-level of 2000 mg/kg/day was toxic).	
Treatment schedule :	one administration daily by the oral route (gavage)
Sampling times:	Day 29; or 24 hours following the single treatment for positive control group.
Solvent & final concentration:	1% carboxymethylcellulose in purified water
Formulation of positive control and final concentration:	cyclophosphamide in distilled water (15 mg/kg) by oral route
Number of sex of animals analysed per group :	5 males & 5 females
Number of cells analysed per animal :	2000 PE (+ 2000 additional PE for the 2nd analysis in males of the higher dose group and control group)

Cytotoxic effects : (see supplementary sheet)

None. For both males & females, the PE/NE ratio in the groups treated was equivalent to those of their respective vehicle control groups, no significant difference was noted.

Genotoxic effects : (see supplementary sheet)

1) For males only, a slight significant increase in the frequency of MPE was noted in the high dose treated group, without any dose-relationship. Therefore, to increase the sample size, an extended analysis was performed in males from the vehicle control and the high dose treated groups.
2) Further to this additional analysis, data show that no significant difference was noted between males treated at 1000 mg/kg/day and the corresponding vehicle control group. These data were combined to the data previously generated and no significant difference was observed. It was therefore concluded that no biological relevance could be attributed to the slight statistically significant increase noted in males following the first analysis and that the test substance did not induce any increase in the frequency of MPE.

Effects of the positive control :

The number of micronucleated cells in the positive control group was significantly higher than in the concurrent vehicle control group.

CONCLUSION: the test substance does not induce damage to the chromosomes or to the mitotic apparatus of rats bone marrow cells after 4-week oral administrations, at the dose-levels of 0, 500 or 1000 mg/kg/day.

Table 16

Table 2 of the report: individual data

	Vehicle		500 mg/kg		1000 mg/kg		CP 15 mg/kg	
	MPE/2000 PE	PE/NE ratio	MPE/2000 PE	PE/NE ratio	MPE/2000 PE	PE/NE ratio	MPE/2000 PE	PE/NE ratio
Male								
1	1	1,7	5	0,7	5	1,1	55	0,6
2	4	1,1	0	1,1	6	0,5	51	0,4
3	2	0,8	3	0,9	3	0,3	37	0,2
4	3	0,5	8	0,8	8	0,9	74	0,3
5	2	1,0	9	1,1	3	0,7	42	0,2
mean/sd	2,40 / 1,14	1,02 / 0,42	5,00 / 3,67	0,91 / 0,17	5,00 / 2,12	0,71 / 0,29	51,80 / 14,31	0,34 / 0,16
Female								
1	1	0,3	4	3	2	1,4	43	0,6
2	5	1,0	4	0,8	7	0,8	15	0,5
3	5	0,6	6	0,9	4	1,3	39	0,4
4	5	1,5	6	1,7	3	0,5	19	0,3
5	2	0,7	2	0,7	5	1,5	42	0,3
mean/sd	3,60 / 1,95	0,82 / 0,46	4,40 / 1,67	1,43 / 0,98	4,20 / 1,92	1,09 / 0,44	31,60 /13,48	0,40 / 0,13

Table 1 of the report: first slide analysis, data summary

	MPE/1000 PE	Statistics	PE/NE ratio	Statistics
Males	mean/sd		mean/sd	
Vehicle	1,2 / 0,6		1,0 / 0,4	
500 mg/kg	2,5 / 1,8		0,9 / 0,2	
1000 mg/kg	2,5 / 1,1	$p < 0,05$	0,7 / 0,3	
CP 15 mg/kg	25,9 / 7,2	$p < 0,001$	0,3 / 0,2	$p < 0,01$
Females				
Vehicle	1,8 / 1,0		0,8 / 0,5	
500 mg/kg	2,2 / 0,8		1,4 / 1,0	
1000 mg/kg	2,1 / 1,0		1,1 / 0,4	
CP 15 mg/kg	15,8 / 6,7	$p < 0,001$	0,4 / 0,1	

Table 3 of the report: second slide analysis, data summary

	Doses	MPE/1000 PE	PE/NE ratio
Males	mg/kg	mean/sd	mean/sd
Vehicle control	-	1,8 / 1,2	0,9 / 0,5
Test substance	1000	2,0 / 1,0	0,6 / 0,4

MPE: Micronucleated Polychromatic Erythrocytes
PE: Polychromatic Erythrocytes
NE: Normochromatic Erythrocytes
sd: standard deviation
CP: cyclophosphamide

Table 5 of the report: pooled data of the 2 slide analysis, data summary

	Doses	MPE/1000 PE
Males	mg/kg	mean/sd
Vehicle control	-	1,5 / 1,2
Test substance	1000	2,3 / 1,0

Statistical tests used:
The 2 x 2 contingency table for MPE, except for the low dose group for males where the Mann-Withney test was used
Student's "t" test for PE/NE ratio

Table 17. Main assay results showing significance in the high-dose in males

Group	Doses (mg/kg/day)	MPE/1000PE mean	(sd)		PE/NE ratio mean	(sd)		Time of sacrifice after the last administration
Males								
Vehicle	-	1.2	*(0.6)*		1.0	*(0.4)*		
	500	2.5	(1.8)		0.9	(0.2)		24 h
Test substance	1000	2.5	(1.1)	*	0.7	(0.3)		
Cyclophosphamide	15 mg/kg	25.9	(7.2)	***	0.3	(0.2)	**	
Females								
Vehicle	-	1.8	(1.0)		0.8	(0.5)		
	500	2.2	(0.8)		1.4	(1.0)		
Test substance	1000	2.1	(1.0)		1.1	(0.4)		24 h
Cyclophosphamide	15 mg/kg	15.8	(6.7)	***	0.4	(0.1)		

Five animals per group

Route: oral
Vehicle: 1% carboxymethylcellulose
Cyclophosphamide: one administration

MPE: Micronucleated Polychromatic Erythrocytes
PE: Polychromatic Erythrocytes
NE: Normochromatic Erythrocytes

Statistical tests used : * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$
The 2 x 2 contingency table for MPE, except for the low dose group for males where the Mann-Withney test was used
Student's "t" test for PE/NE ratio

In conclusion, the micronucleus test was positive in males at a high dose of 1000 mg/kg/day after 4 weeks of treatment, but the sponsor concludes it was negative because of the absence of a dose-response effect.

ADDENDUM:
Although the initial study showed a small, but significant, increase in MPCE in males, an extended analysis to increase the sample size was performed in the males in the vehicle control and the high dose treated groups. Data generated in this additional analysis showed that no significant difference was noted between the groups. These data were combined to the previous data in order to increase the size of the samples, and no significant differences between groups were observed. Data are shown in the table below (taken from the Sponsor):

Table 6: Results of the cytogenetic test: pooled data, individual values

Pooled data

		Vehicle			Test substance (1000 mg/kg/day)	
sex	slide	MPE/4000PE	sex	slide	MPE/4000PE
Male	07	3	Male	18	10
	37*	5		05	9
	08	6		39	4
	16	5		20	14
	35	9		02	8
mean MPE/4000PE		5.89	mean		9.00
sd		2.19	sd		3.61

Thus, upon further evaluation of the data presented, it is the opinion of this Reviewer that carglumic acid, at the doses tested (500 and 1000 mg/kg/day), was not positive in the *in vivo* micronucleus assay after a 4-week treatment in the rats. The revised conclusion is based on: 1) There was no dose-dependency in the increase in MPE frequency after carglumic acid treatment at 500 and 1000 mg/kg/day; 2) Extended analysis of the samples revealed no statistically significant increase in MPE frequency after 4 weeks of carglumic acid treatment.

The overall result from the three *in vivo* micronucleus studies showed a positive micronucleus result only in the first study, in which a less pure batch of carglumic acid (0.3% HPA as compared to 0.1% HPA in the subsequent batch) was used. It is concluded that carglumic acid does not induce micronuclei in rat bone marrow cells at the tested doses of 500 and 1000 mg/kg/day.

Effects of HPA (hydantoin-5-propionic acid, the impurity of carbamoyl glutamate) on Chromosome Aberrations in Cultured Human Peripheral Blood Lymphocytes (HPBL).

Study no: (b) (4)-R980906

Volume #, and page #: amendment 004, submission 1/13/04, page 001

Conducting laboratory and location: (b) (4)
(b) (4)

Date of study initiation: 3/17/1998

GLP compliance: Yes

QA reports: yes (X) no ( )

Drug lot #, and % purity: Batch #: 03149806R21 of hydantoin-5-propionic acid (HPA).

Formulation/vehicle: RPMI 1640 cell culture medium. HPA was prepared in RPMI 1640 at a concentration of 50 mg/ml. This solution was added at 10% to the culture medium, giving a final concentration of 5,000 µg/ml. A pH adjustment was made before treatment using 7.5% sodium hydrocarbonate solution.

Methods:
Test strain and Cells: Cultured human peripheral blood lymphocytes (HPBL) were obtained from blood collected from two healthy donors (1M+1F).

Dose selection criteria:
Basis of dose selection: The dose selection was based on the mitotic index which was used to measure the cytotoxic/cytostatic effect by analyzing 1000 cells from each culture. The mitotic index (MI) reduction was evaluated compared to the negative controls.

Range finding studies:

In the absence of metabolic activation: The dose range was 313-5000 µg/ml. In the first assay with a short treatment (4 hours + 20 hours of sampling time) using the above dose range of 313-5000 µg/ml, cytotoxicity (MI was 78% vs control) was observed at 5000 µg/ml, therefore 5000 µg/ml was selected as a high dose in this assay. Following continuous treatment in the preliminary assay at 2500 µg/ml, MI was 56% at 20 hours and 76% at 44 hours. Therefore, in the second assay for continuous treatment, the highest doses of HPA selected were 3000 and 4000 µg/ml without metabolic activation at 20 and 44 hours respectively. However in the second assay (without metabolic activation) higher cytotoxicity was observed, therefore the maximum doses used for scoring were 2500 and 3500 µg/ml, respectively.

In the presence of metabolic activation: Using either 5% S9 mix or 10% S9 mix, 5000 µg/ml was selected as a top dose for both assays, because a slight increase in cytotoxicity was noted at this dose (MI was decreased to 81% of control).

Test agent stability: The stability data were not provided.

Metabolic Activation System: Rat liver microsome S9 fraction.

Controls:

Vehicle or negative controls: RPMI-1640 culture medium

Positive controls: Mitomycin C 0.25 µg/ml (for system without S9), Cyclophosphamide (CP, 10 µg/ml for system with S9).

Exposure conditions/Study design: This assay determines clastogenesis (chromosomal aberrations) in an *in vitro* assay. Replicate HPBL cells were exposed to various concentration of HPA. Two assays were performed. Positive and negative controls were similarly treated. The scoring of aberrations was performed on 100 metaphase cells.

Doses used in definitive study: In the first assay, 1250, 2500, and 5000 µg/ml were used with or without S9 (treatment for 4 hours, sampling at 20 hours). In the second assay without S9, dose levels of 1500, 2000, and 2500 µg/ml were used for 20-hr treatment; 2500, 3000, and 3500 µg/ml were used for 44-hr treatment. In the second assay with S9, dose levels of 1250, 2500, and 5000 µg/ml (treatment for 4 hours, sampling at 20 hours) were used. At these doses, no significant increase in osmolality was observed (312, 300, 307, 308 mOsmol/kg at 0, 1250, 2500, and 5000 µg/ml respectively). The pH values are shown below.

	Dose	pH Values					
		Before pH correction	After pH correction*	End of treatment			
				S9- (4h)	S9- (20 h)**	S9- (44 h)**	S9+
Solvent control	0	7,4	7,94	7,83	8,01	7,44	7,61
Test compound	5000	5,12	6,51	6,61	7,12	6,85	6,54
	2500	6,31	6,99	7,16	7,67	7,27	7,05
	1250	6,9	7,42	7,5	N.T.	N.T.	7,37

* Before treatment ** in cytotoxicity assay NT: not tested

Analysis:
Counting method: At the end of the study, 100 cells in metaphase/culture were examined for structural aberrations. In the positive controls, 50 metaphase cells/culture were examined.

Criteria for positive results: HPA would be considered positive if: the percentage of cells with aberrations (excluding gaps) is increased in a dose-related manner; a statistically significant increase in relation to the vehicle or negative control is demonstrated; the values in the negative control are in the range of the historical solvent control; the increase in the positive control was statistically significant.

Summary of individual study findings:
Study validity: Appropriate dose selection was made for this study based on toxicity, and positive control responses were acceptable.

Study outcome: Two assays were performed. In the first assay (Expt.1), the tested dose levels were 1250, 2000, 2500, and 5000 µg/ml, the treatment time was approximately 4 hours, and harvest time was 20 hours after initiation of the treatment (with and without metabolic activation. In the second assay, treatment with 1500, 2000, and 2500 µg/ml for 20 hr was used without S9. Results showed a statistically significant increase in the number of breaks/cell, and in the frequency of abnormal cells (excluding gaps only) at 4 hours and 20 hours treatment in the absence of metabolic activation in both assays. The number of gaps/cell was increased at 1250-2500 µg/ml (see sponsor's Tables 8 and 12). The mitotic index (or cytotoxicity data) in the preliminary assay was 93%, 94%, 87%, 98%, and 78*% of control at 313, 625, 1250, 2500, and 5000 µg/ml, respectively (*$p<0.01$) in HPBL in the absence of S9 at 4 hours. The mitotic index in the second assay was 100%, 97%, 95%, 56%* and 1%* of control at 313, 625, 1250, 2500, and 5000 µg/ml respectively (*$p<0.001$) in the absence of S9 at 20 hours. The dose related trend could be seen in figures 1A and 1B. However, no changes in aberrations were observed

in the presence of metabolic activation in both assays. The combined data on both assays are presented in Tables A and B.

Sponsor's Table 8. Data from 4-hr treatment showing dose-related increases in chromosome aberrations in HPBL (expt. 1) in the absence of metabolic activation.

TABLE 8

IN VITRO HUMAN LYMPHOCYTE METAPHASE ANALYSIS
RESEARCH WITHOUT METABOLIC ACTIVATION

TEST COMPOUND HYDANTOIN-5-PROPIONIC ACID
SPONSOR ORPHAN EUROPE

ASSAY 1
Short treatment: 4 hours
Sampling time: 20 hours after the start of treatment

COMPOUND	DOSES µg/ml		GAPS PER CELL	BREAKS PER CELL	NUMBER OF ABERRANT CELLS/ NUMBER OF CELLS OBSERVED	TOTAL OF NUMERICAL ABERRATIONS	TOTAL OF CELLS CARRYING STRUCTURAL ABERRATIONS	
							EXCLUDING CELLS WITH GAPS ONLY	INCLUDING CELLS WITH GAPS ONLY
SOLVENT CONTROLS	0	m	0.005	0.025	No. ABERRANT CELLS	0	5	6
		s	0.071	0.157	No. CELLS OBSERVED	200	200	200
MITOMYCIN C	0.25	m	0.12	0.78	No. ABERRANT CELLS	0	57	60
		s	0.356	1.263	No. CELLS OBSERVED	100	100	100
		t	3.199	7.279	χ^2	0	120.775	126.224
		p	<0.01	<0.001	p	N.S.	<0.001	<0.001
TEST COMPOUND	1250	m	0.05	0.01	No. ABERRANT CELLS	0	2	12
		s	0.218	0.1	No. CELLS OBSERVED	200	200	200
		t	2.776	1.14	χ^2	0	0.582	2.094
		p	<0.01	N.S.	p	N.S.	N.S.	N.S.
	2500	m	0.045	0.025	No. ABERRANT CELLS	0	5	12
		s	0.252	0.157	No. CELLS OBSERVED	200	200	200
		t	2.161	0	χ^2	0	0	2.094
		p	<0.05	N.S.	p	N.S.	N.S.	N.S.
	5000	m	0.025	0.155	No. ABERRANT CELLS	1	17	19
		s	0.186	0.681	No. CELLS OBSERVED	200	200	200
		t	1.421	2.631	χ^2	0	6.926	7.211
		p	N.S.	<0.01	p	N.S.	<0.01	<0.01

m=mean ; s=standard deviation ; t= Student's t ; N.S.=not statistically significant on the threshold of p=0.05

Sponsor's Table 12. Data from 20-hr treatment showing dose-related increases in chromosome aberrations in HPBL (expt. 2) in the absence of metabolic activation.

RESEARCH WITHOUT METABOLIC ACTIVATION

COMPOUND HYDANTOIN-5-PROPIONIC ACID
SPONSOR ORPHAN EUROPE

ASSAY 2
Continuous treatment: 20 hours
Sampling time : at the end of treatment

COMPOUND	DOSES µg/ml		GAPS PER CELL	BREAKS PER CELL	NUMBER OF ABERRANT CELLS/ NUMBER OF CELLS OBSERVED	TOTAL OF NUMERICAL ABERRATIONS	TOTAL OF CELLS CARRYING STRUCTURAL ABERRATIONS	
							EXCLUDING CELLS WITH GAPS ONLY	INCLUDING CELLS WITH GAPS ONLY
SOLVENT CONTROLS	0	m	0	0.005	No. ABERRANT CELLS	0	1	1
		s	0	0.071	No. CELLS OBSERVED	200	200	200
MITOMYCIN C	0.25	m	0.13	1.62	No. ABERRANT CELLS	0	64	67
		s	0.367	1.728	No. CELLS OBSERVED	100	100	100
		t	3.542	9.342	χ^2	0	158.386	168.189
		p	<0.001	<0.001	p	N.S.	<0.001	<0.001
TEST COMPOUND	1500	m	0.015	0.01	No. ABERRANT CELLS	0	2	5
		s	0.122	0.1	No. CELLS OBSERVED	200	200	200
		t	1.739	0.577	χ^2	0	0	1.523
		p	N.S.	N.S.	p	N.S.	N.S.	N.S.
	2000	m	0.03	0.03	No. ABERRANT CELLS	0	5	11
		s	0.171	0.198	No. CELLS OBSERVED	200	200	200
		t	2.481	1.681	χ^2	0	1.523	6.959
		p	<0.05	N.S.	p	N.S.	N.S.	<0.01
	2500	m	0.04	0.07	No. ABERRANT CELLS	1	12	19
		s	0.196	0.292	No. CELLS OBSERVED	200	200	200
		t	2.886	3.059	χ^2	0	7.951	15.211
		p	<0.01	<0.01	p	N.S.	<0.01	<0.001

m=mean ; s=standard deviation ; t= Student's t ; N.S.=not statistically significant on the threshold of p=0.05

Table A: Combined data in assay 1 and assay 2 without metabolic activation are shown below.

RESEARCH WITHOUT METABOLIC ACTIVATION

RECAPITULATION OF 2 ASSAYS

TEST COMPOUND : HYDANTOIN-5-PROPIONIC ACID

SPONSOR : ORPHAN EUROPE

ASSAY 1	SHORT TREATMENT: 4 HOURS			ASSAY 2	CONTINUOUS TREATMENT: 20 HOURS			ASSAY 2	CONTINUOUS TREATMENT: 44 HOURS		
	Sampling time: 20 h after the start of treatment				Sampling time: at the end of treatment				Sampling time: at the end of treatment		
	STRUCTURAL ABERRATIONS				STRUCTURAL ABERRATIONS				STRUCTURAL ABERRATIONS		
DOSES µg/ml	Break per cell	Abnormal cells excluding gaps only n=200	Abnormal cells including gaps only n=200	DOSES µg/ml	Break per cell	Abnormal cells excluding gaps only n=200	Abnormal cells including gaps only n=200	DOSES µg/ml	Break per cell	Abnormal cells excluding gaps only n=200	Abnormal cells including gaps only n=200
Controls 0	0.025 ±0.157	5	6	Controls 0	0.005 ±0,071	1	1	Controls 0	0.025 ±0,157	5	9
1250	0.01 ±0.1 N.S.	2 N.S.	12 N.S.	1500	0.01 ±0,1 N.S.	2 N.S.	5 N.S.	2500	0.01 ±0,1 N.S.	2 N.S.	10 N.S.
2500	0.025 ±0.157 N.S.	5 N.S.	12 N.S.	2000	0.03 ±0,198 N.S.	5 N.S.	11 <0.01	3000	0.05 ±0,218 N.S.	10 N.S.	18 N.S.
5000	0.155 ±0.681 <0.01	17 <0.01	19 <0.01	2500	0.07 ±0,292 <0.01	12 <0.01	19 <0.001	3500	0.035 ±0,184 N.S.	7 N.S.	16 N.S.

N.S.: not statistically significant on the threshold of p=0.05
n= number of cells scored per dose
Student's t-test for break per cell
χ^2 test for damaged cells with and without gaps

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Table B: Combined data in assay 1 and assay 2 with metabolic activation are shown below.

RESEARCH WITH METABOLIC ACTIVATION

RECAPITULATION OF 2 ASSAYS

TEST COMPOUND : HYDANTOIN-5-PROPIONIC ACID

SPONSOR : ORPHAN EUROPE

ASSAY 1	SHORT TREATMENT: 4 HOURS 5% S9-mix			ASSAY 2	SHORT TREATMENT: 4 HOURS 10% S9-mix		
	Sampling time: 20 h after the start of treatment				Sampling time: 20 h after the start of treatment		
	STRUCTURAL ABERRATIONS				STRUCTURAL ABERRATIONS		
DOSES µg/ml	Break per cell	Abnormal cells excluding gaps only n=200	Abnormal cells including gaps only n=200	DOSES µg/ml	Break per cell	Abnormal cells excluding gaps only n=200	Abnormal cells including gaps only n=200
Controls 0	0.01 ±0.1	2	5	Controls 0	0.01 ±0.1	2	5
1250	0.02 ±0.14 N.S.	4 N.S.	8 N.S.	1250	0.01 ±0.1 N.S.	2 N.S.	2 N.S.
2500	0.01 ±0.1 N.S.	2 N.S.	3 N.S.	2500	0.02 ±0.14 N.S.	4 N.S.	5 N.S.
5000	0.015 ±0.122 N.S.	3 N.S.	5 N.S.	5000	0 ±0 N.S.	0 N.S.	2 N.S.

N.S.: not statistically significant on the threshold of p=0.05
n= number of cells scored per dose
Student's t-test for break per cell
$\chi 2$ test for damaged cells with and without gaps

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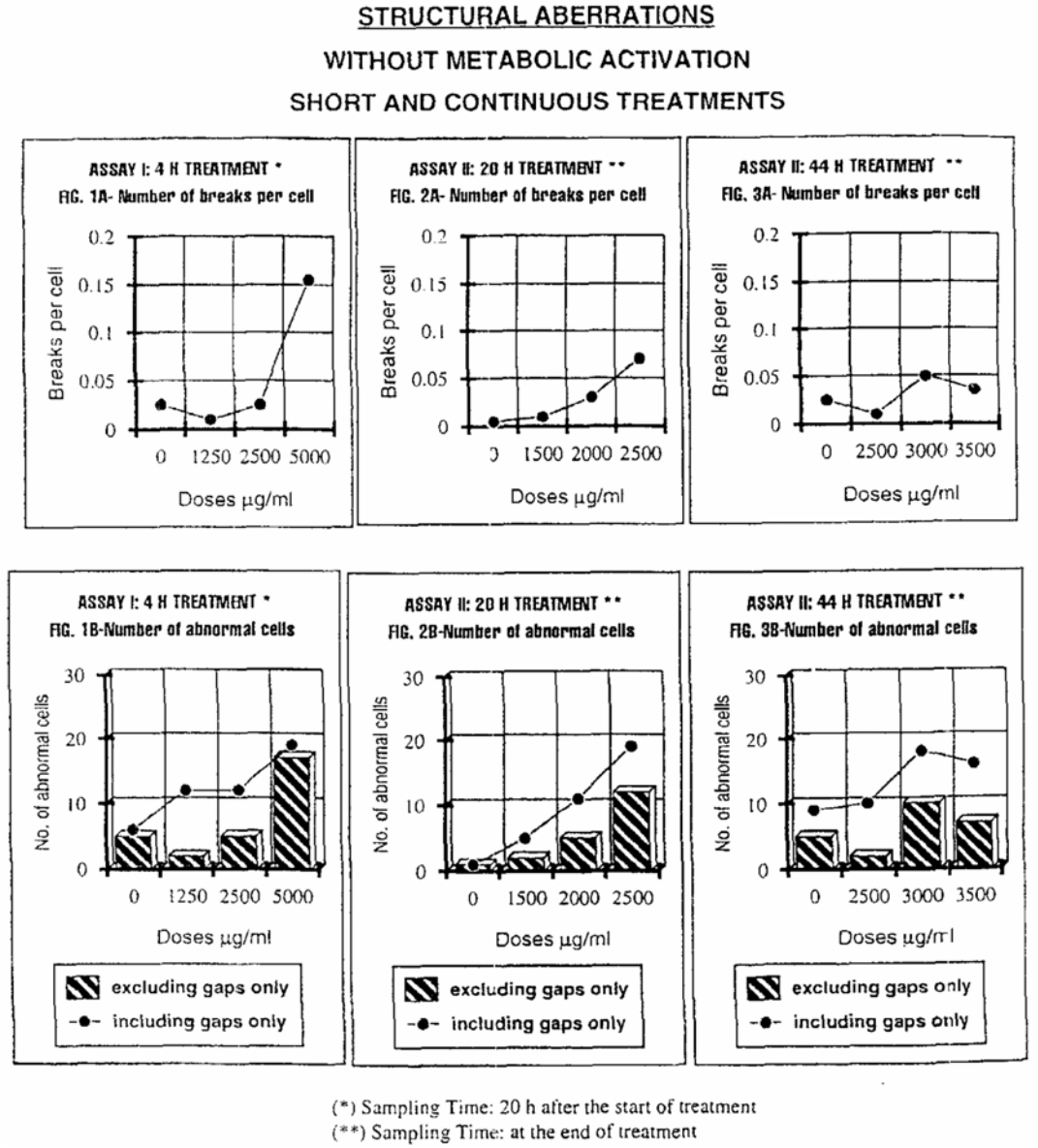
Figures: The structural aberrations are shown below. See the dose-response effect in figure 1A and 1B (at 4 hours in assay 1) and in 2A and 2 B (at 20 hours in assay 2).



(*) Sampling Time: 20 h after the start of treatment
(**) Sampling Time: at the end of treatment

The following is Sponsor's conclusion in this assay:

Without metabolic activation in the assays using 4 hour treatment (Table 8, page 30) and 20 hour treatment (Table 12, page 36) at the highest doses tested of 5000 and 2500 µg/ml respectively, a statistically significant increase in the frequency of numerical or structural chromosomal aberrations was noted: breaks per cell (0.155 vs 0.025 and 0.07 vs 0.005), abnormal cells excluding gaps only (17 vs 5 and 12 vs 1) and including gaps only (19 vs 6 and 19 vs 1). In addition, this effect was dose-related (Figures 1A-1B and 2A-2B, page 45). It is noted that in the 20 hour treatment assay, at the intermediary dose of 2000 µg/ml, a significant increase in the frequency of abnormal cells including gaps only was observed alone. In return, no statistically significant increase in the number of breaks per cell or in the frequency of abnormal cells excluding and including gaps only was seen in the assay using 44 hour treatment (Table 14, page 39 and Figures 3A-3B, page 45).

With metabolic activation, both in the assays using 5% (Table 10, page 33 and Figures 4A-4B, page 46) and 10% S9-mix (Table 16, page 42 and Figures 5A-5B, page 46), no statistically significant increase in the frequency of numerical or structural chromosomal aberrations was observed at any dose tested.

In conclusion, under these experimental conditions, **the compound HYDANTOIN-5-PROPIONIC ACID provided by ORPHAN EUROPE induced a clastogenic activity without metabolic activation in the *in vitro* human lymphocyte metaphase analysis test.**

Thus in both assays, the number of breaks and number of abnormal cells were increased at all doses in a dose related manner (figures 1A, 1B, 2A, 2B). The positive controls (with or without S9 mix) showed a significant increase in aberrations. In conclusion, HPA was clastogenic in both the initial and the confirmatory assays when HPA exposure was 4 hours (Expt 1) or 20 hours (Expt 2), in the absence of S9. with acceptable cytotoxicity. The test was negative in the presence of S9.

Summary: HPA (a major impurity in carglumic acid) tested positive in the absence of metabolic activation in the chromosome aberration test in cultured human peripheral blood lymphocytes,

Bacterial Mutagenicity Test on Salmonella Typhimurium HIS (5 strains) Using B.N. Ames's Technique with Diaza-1, 3-dione-2, 4-carboxy-7-cycloheptane

Key findings: Diaza-1, 3-dione-2, 4-carboxy-7-cycloheptane was negative in the Ames assay.

Study no.: FSR (b) (4) 081001

Volume #, and page #: Vol. 1.12, page 1-42

Conducting laboratory and location: (b) (4)

Date of study initiation: 01/13/2009

GLP compliance: Yes

QA reports: yes (x) no ( )

Drug, lot #, and % purity: Diaza-1, 3-dione-2, 4-carboxy-7-cycloheptane; JT-AC-T164; >99.4%

Methods

Strains/species/cell line: *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537, and TA102

Doses used in definitive study: 0, 50, 150, 500, 1500, and 5000 µg/plate

Dose selection criteria:
Basis of dose selection: Based on negative results in the cytotoxicity assay.

Range finding studies: Doses of 50-5000 µg/plate were used in all strains in the presence and absence of metabolic activation, and no cytotoxicity or precipitate was noted at the highest dose.

Test agent stability: Not stated.

Metabolic activation system: Rat liver microsome S9 fraction.

Controls:
Vehicle or negative controls: Phosphate buffer

Positive controls:

4.7. Reference products used for the positive controls

Strains	Without metabolic activation		With metabolic activation		
	Substance	Dose µg/plate	Substance	without pre-incubation Dose µg/plate	with pre-incubation Dose µg/plate
TA1535	sodium azide	1	2-anthramine	2	1
TA1537	9-amino-acridine	50	2-anthramine	2	1
TA98	2-nitro fluorene	2	2-anthramine	2	1
TA100	sodium azide	1	2-anthramine	2	1
TA102	mitomycin C	0.125	benzo[a]pyrene	2	2

Comments:
Exposure conditions/Study design: The preincubation method (48 hours) was used. The tester strains in the plate (in duplicate cultures) were exposed to the vehicle, drug, or positive controls.

Analysis:
No. of replicates: Triplicate cultures/dose

Counting method: The colonies were counted manually or automatically, using a colony counter.

Criteria for positive results: If Diaza-1, 3-dione-2, 4-carboxy-7-cycloheptane induces an increase in revertant colonies in a dose-dependent manner, and the increase is at least 2 times for strains TA98, TA100, and TA102, and 3 times for strains TA1535 and TA1537 compared to vehicle controls, it would be considered positive.

Results:

Study validity: Appropriate dose selection was made for this study, and positive control responses were acceptable.

Study outcome: Two independent experiments were carried out. Diaza-1, 3-dione-2, 4-carboxy-7-cycloheptane was not mutagenic in any of the tester strains at doses ranging from 50-5000 µg/plate in the presence or absence of metabolic activation. However, a significant increase in the number of revertant colonies was observed with the positive controls (with or without S9 mix). The results are shown in the tables below (taken from study report).

Test item: **Diaza-1,3-dione-2,4-carboxy-7-cycloheptane** Solvent : **Phosphate buffer**

	TA 1535			TA 1537			TA 98			TA 100			TA102		
	DOSES in µg/plate	revertants /plate	Induction Ratio (a)	DOSES in µg/plate	revertants /plate	Induction Ratio (a)	DOSES in µg/plate	revertants /plate	Induction Ratio (a)	DOSES in µg/plate	revertants /plate	Induction Ratio (a)	DOSES in µg/plate	revertants /plate	Induction Ratio (a)
Positive control	(b)	236.7	**16.1**	(b)	534.7	**106.9**	(b)	216.7	**15.2**	(b)	460.0	**4.9**	(b)	1530.7	**9.1**
	0	14.0	-	0	5.5	-	0	14.0	-	0	93.0	-	0	157.0	-
TEST ITEM	50	20.3	**1.5**	50	5.3	**1.0**	50	16.7	**1.2**	50	114.3	**1.2**	50	181.3	**1.2**
	150	19.7	**1.4**	150	4.3	**0.8**	150	17.3	**1.2**	150	113.7	**1.2**	150	169.3	**1.1**
WITHOUT	500	23.3	**1.7**	500	5.7	**1.0**	500	11.7	**0.8**	500	103.3	**1.1**	500	178.0	**1.1**
S9-mix	1500	22.3	**1.6**	1500	5.3	**1.0**	1500	17.7	**1.3**	1500	108.7	**1.2**	1500	168.7	**1.1**
	5000	31.7	**2.3**	5000	4.7	**0.9**	5000	19.7	**1.4**	5000	101.7	**1.1**	5000	184.0	**1.2**
Positive control	(c)	312.7	**44.7**	(c)	222.7	**67.5**	(c)	2066.7	**121.6**	(c)	2178.7	**22.1**	(c)	1061.3	**3.5**
	0	8.7	-	0	5.0	-	0	23.0	-	0	105.3	-	0	262.0	-
TEST ITEM	50	7.7	**0.9**	50	8.7	**1.7**	50	24.3	**1.1**	50	118.7	**1.1**	50	344.7	**1.3**
	150	9.0	**1.0**	150	6.7	**1.3**	150	26.0	**1.1**	150	132.0	**1.3**	150	351.3	**1.3**
WITH	500	11.3	**1.3**	500	5.3	**1.1**	500	17.3	**0.8**	500	129.3	**1.2**	500	386.0	**1.5**
S9-mix	1500	10.3	**1.2**	1500	9.7	**1.9**	1500	23.3	**1.0**	1500	117.3	**1.1**	1500	402.0	**1.5**
	5000	7.3	**0.8**	5000	6.3	**1.3**	5000	17.7	**0.8**	5000	111.3	**1.1**	5000	328.7	**1.3**

(a) Induction Ratio = number of revertants in the treated / number of revertants in the control
Reference positive compounds (µg/plate):
(b) TA1535 and TA100: Sodium azide[w] 1; TA1537: 9-amino-acridine 50; TA98: 2-nitrofluorene[d] 2; TA102: Mitomycin C[w] 0.125
(c) TA1535, TA1537, TA98, TA100: 2-anthramine[d] 2; TA102: benzo(a)pyrene[d] 2
Solvents used for positive controls: [d] DMSO; [w] distilled water

Test item: Diaza-1,3-dione-2,4-carboxy-7-cycloheptane Solvent : Phosphate buffer

	TA 1535			TA 1537			TA 98			TA 100			TA102		
	DOSES in µg/plate	revertants /plate	Induction Ratio (a)	DOSES in µg/plate	revertants /plate	Induction Ratio (a)	DOSES in µg/plate	revertants /plate	Induction Ratio (a)	DOSES in µg/plate	revertants /plate	Induction Ratio (a)	DOSES in µg/plate	revertants /plate	Induction Ratio (a)
Positive control	(b)	378.7	17.0	(b)	1162.0	184.4	(b)	242.7	20.7	(b)	682.7	6.4	(b)	1525.3	6.7
TEST ITEM	0	26.0	-	0	6.7	-	0	17.8	-	0	112.0	-	0	203.3	-
	50	21.0	0.8	50	6.0	0.9	50	14.0	0.8	50	115.0	1.0	50	207.3	1.0
	150	26.0	1.0	150	6.3	0.9	150	21.3	1.2	150	134.3	1.2	150	184.0	0.9
WITHOUT	500	30.7	1.2	500	6.3	0.9	500	12.0	0.7	500	142.0	1.3	500	228.0	1.1
S9-mix	1500	21.0	0.8	1500	6.0	0.9	1500	14.7	0.8	1500	136.7	1.2	1500	240.0	1.2
	5000	23.0	0.9	5000	4.7	0.7	5000	16.0	0.9	5000	113.7	1.0	5000	221.3	1.1
Positive control	(c)	184.0	21.1	(c)	182.0	15.2	(c)	2832.0	127.0	(c)	2005.3	23.3	(c)	664.0	1.8
TEST ITEM	0	11.3	-	0	8.0	-	0	28.8	-	0	89.8	-	0	293.0	-
	50	12.3	1.1	50	7.3	0.9	50	24.0	0.8	50	93.7	1.0	50	319.3	1.1
WITH	150	12.3	1.1	150	7.7	1.0	150	26.0	0.9	150	120.0	1.3	150	321.3	1.1
S9-mix	500	11.7	1.0	500	6.3	0.8	500	29.0	1.0	500	106.7	1.2	500	344.0	1.2
With pre-	1500	14.3	1.3	1500	7.3	0.9	1500	19.3	0.7	1500	108.3	1.2	1500	340.0	1.2
incubation	5000	12.0	1.1	5000	6.7	0.8	5000	20.0	0.7	5000	89.0	1.0	5000	356.0	1.2

Conclusion: Diaza-1, 3-dione-2, 4-carboxy-7-cycloheptane, a potential impurity in carglumic acid, was not mutagenic in the Ames assay either in the presence or absence of metabolic activation, in two independent assays.

Study of Genotoxic Activity Using the Micronucleus Test in Mice with the Test Item Hydantoin-5-Propionic acid

Study no.: FSR (b) (4) 081003

Volume #, and page #: Vol. 1.12, page 1-39

Conducting laboratory and location: (b) (4)
Date of study initiation: 01/20/2009

GLP compliance: Yes

QA reports: yes (x) no ( )

Drug, lot #, and % purity: hydantoin-5-propionic acid; JT-AC-T151; >99.4%

Formulation/vehicle: Phosphate buffer

Methods:

Test strain: OF1 mice (b) (4)), 5 males/dose (26.1 to 29.7 g) and 5 females/dose (20 to 26 g)
Doses used in definitive study: 312.5, 625, and 1250 mg/kg/day in males and 500, 1000, and 2000 mg/kg/day in females.

Basis of dose selection: In the preliminary toxicity assay, the dose of 2000 mg/kg/day (x2) elicited convulsion in males 10 minutes after the second treatment. One of two males died after the second treatment. The lower dose of 1250 mg/kg/day (x2) induced neither mortality nor clinical signs. In females, the doses of 1250 and 2000 mg/kg/day

(x2) elicited neither clinical signs nor mortality. Thus, doses of 1250 and 2000 mg/kg/day (x2) were selected as the high dose for males and females, respectively, in the confirmatory assay.

Test agent stability: Not stated.

Controls:
Vehicle or negative controls: phosphate buffer

Positive controls: Cyclophosphamide (CP), 50 mg/kg by IP route.

Exposure conditions/Study design: In the main study, 5 male were treated with 312.5, 625, and 1250 mg/kg/day (x2) for 2 days and 5 female were treated with 500, 1000, and 2000 mg/kg/day (x2) for 2 days. A group of mice was similarly treated with the vehicle (phosphate buffer) or positive control (CP). Animals were sacrificed at 24 hours after the last treatment, and bone marrow cells were prepared.

Counting method: Polychromatic erythrocytes (PCE)/normochromatic erythrocytes (NCE) ratio was determined by analyzing at least 1000 erythrocytes per animal. Micronucleated polychromatic erythrocytes (MPE) were counted in 2000 polychromatic erythrocytes.

Criteria for positive results: For a test item to be considered positive in the micronucleus test, there must be a statistically significant increase in the number of micronuclei, with at least a 2-fold increase in micronuclei compared with the negative control animals at one of three doses tested. For a test item to be considered negative in the micronucleus test, there must be no statistically significant increase in the number of micronuclei observed compared with negative control animals.

Results

Study validity: Appropriate dose selection was made for this study based on toxicity data, and positive control responses were acceptable.

Study outcome: The results obtained on negative control animals and those treated with the positive reference substance were similar to historic values. A statistically significant increase in the frequency of micronuclei was noted in the group treated with cyclophosphamide, demonstrating the sensitivity of the assay. No statistically significant increase in the frequencies of MPE was found in the animals treated with HPA at any dose, in males or females separately, or in both sexes combined, when compared with the control group (see table below). A statistically significant decrease in the number of MPE was noted in the 625 mg/kg/day male group when compared to the vehicle control; this decrease has no meaning in terms of genotoxic potential.

SAMPLING TIME (24 H after last treatment)	TEST ITEM DOSES in mg/kg/day (x2)	SEX	PCE / NCE RATIO		MICRONUCLEI FOR 1000 PCE	
			Mean	Student's t Test (p)	Mean	Mann Whitney (p)
Negative control Group	VEHICLE 20 mL/kg (female) - 12.5 mL/kg (male)	M F M + F	*2.47* *3.30* *2.89*		*0.90* *0.30* *0.60*	
Positive control Group	Cyclophosphamide 50 mg/kg/day (x1)	M F M + F	*2.41* *2.10* *2.25*	**N.S.** **N.S.** **N.S.**	*13.00* *14.30* *13.65*	**p<0.01** **p<0.01** **p<0.001**
Hydantoin-5-propionic acid **TREATED** **GROUPS**	**1250** **2000**	M F M + F	*1.76* *1.73* -	**<0.05** **<0.05** -	*0.60* *0.50* -	**N.S.** **N.S.** -
	625 **1000**	M F M + F	*1.89* *1.83* -	**N.S.** **<0.05** -	*0.30* *0.10* -	**p<0.05** **N.S.** -
	312.5 **500**	M F M + F	*2.24* *2.34* -	**N.S.** **N.S.** -	*0.50* *0.20* -	**N.S.** **N.S.** -

Conclusion: HPA was negative in the *in vivo* micronucleus assay under the experimental conditions.

Overall summary:
The following table summarizes the results of the genotoxicity studies:

Test Article	AMES	Chromsomal Aberration	Micronucleus assay (24 and 48 hr)	Micronucleus assay 4 week
Carglumic acid (0.3% HPA)	–	+	+	
Carglumic acid (0.1% HPA)		–	–	–
Carglumic acid (pH not adjusted/pH adjusted)		+/–		
HPA	–	+	–	
Diaza cycloheptane	–			

These studies demonstrated that carglumic acid is negative in the Ames test and is not clastogenic in the *in vitro* chromosome aberration assay in human peripheral blood lymphocytes, when pH of the testing medium was neutralized. Carglumic acid containing low levels of HPA (0.1%) impurity is also negative for clastogenic activity in the *in vivo* micronucleus assay in rats. However, HPA is clastogenic in the lymphocyte aberration assay. The totality of the data indicates that carglumic acid lacks genotoxic

activity, and that HPA and/or a change in pH are factors in the clastogenic responses observed in some of the genotoxicity assays.

2.6.6.5 Carcinogenicity

No studies were submitted.

2.6.6.6 Reproductive and Developmental Toxicology

Effects on Fertility and Embryo-fetal Development by Oral Route (gavage) in Female Rats

Study no: 23288 RSR

Volume #, and page #: Submission1/12/05 (# 008), volume 5/8, pg 1.

Conducting laboratory and location: (b) (4).

Date of study initiation: 5/13/02

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, radiolabel, and % purity: First receipt Batch # 05031011P63, second receipt Batch # 05031011P105. Purity data were provided.
Formulation/vehicle: 1% carboxy-menthylcellulose aqueous solution in purified water.

Methods:

Species/strain: Rats, Crl: CD (SD) IGS BR

Doses employed: 0, 500, and 2000 mg/kg/day. Dose selection was based on existing toxicity data.

Route of administration: Oral, 1 ml/kg/day

Study design: The males were used only for mating and did not receive the drug. Females were given the drug 15 days prior to mating, during mating and up to day 17 of gestation and sacrificed on day 20.

Number/sex/group: 24 females/dose; 24 untreated males

See the study design, parameters and endpoints evaluated:

Two groups of 24 female Sprague-Dawley rats received the test item at 500 or 2000 mg/kg/day by daily oral gavage throughout the premating period (15 days), mating period and during pregnancy, until day 17 *post-coitum*.
A group of 24 females was given the vehicle alone (1% carboxymethycellulose in water) and acted as a control group.
Twenty four male rats were used for mating purposes only and did not receive the treatment.
In addition, satellite animals (six females in each treated group) were used to assess the plasma levels of the test item during gestation.
Mortality was checked twice daily and clinical signs were checked daily. Body weight and food consumption were recorded at designated intervals.
A mating trial was carried out on all animals: males and females were paired for a maximum of 14 days. Mating index and pre-coital interval were calculated.
At hysterectomy of the mated females (on day 20 *post-coitum*), the number of corpora lutea, implantation sites, resorptions, dead and live fetuses was recorded. The pre- and post-implantation losses were calculated. The fetuses were weighed, sexed and submitted to external examination. A detailed soft tissue examination was performed on half of the fetuses by serial sectioning after fixation. The remaining half of the fetuses were submitted to a detailed examination of the skeleton following alizarin staining.
Parent females were submitted to a detailed macroscopic examination.
Fertility and gestation indexes were calculated.

Results:

Mortality: Two females in the 2000 mg/kg/day group were found dead. One was found dead on day 4 of the premating period, without any clinical signs. At necropsy, foamy contents in the trachea were observed, therefore the death was considered to be the result of dosing accident. The other female was sacrificed on day 12 of premating period. This animal had poor clinical condition (swollen neck and chromorhinorrhea) and perforation of esophagus. This death was not considered as drug related.

Clinical signs: No drug-related effects were seen except ptyalism (excessive secretion of saliva) at 2000 mg/kg/day after day 7 of treatment.

Body weights/weight gains: Net bodyweight gain was significantly decreased at 2000 mg/kg/day on GD 0-20 (71.9, 71.9, and 59.4* g at 0, 500, and 2000 mg/kg/day, respectively *$p<0.05$). Bodyweights were not affected during the premating period.

Food consumption: Food intake was lower during premating and pregnancy by 4-5% at 2000 mg/kg/day. The mating and fertility indices were not affected by the drug treatment as shown below.

Table. Study design and results

3.5.1 Reproduction Toxicity - Fertility and Embryofetal Development

Report Title: Study for effect on fertility and embryo-fetal development by oral route (gavage) in female rats

Test article: N-carbamyl-L-glutamic acid
Study No. 23288 RSR
Report: volume: 1, pages: 297
Test facility: (b) (4)
GLP compliance: Yes

Design similar to ICH 4.1.1: Yes
Species/Strain: Sprague-Dawley/rat
Initial Age: Male: 10 weeks/Female: 8 weeks
Date of First Dose: 12 May 2002

Duration of Dosing: 15 days before mating, during mating period, during pregnancy until day 17 *post-coitum* inclusive.
Day of Mating: GD0
Day of Hysterectomy: GD20
Method of Administration: oral route (gavage)
Vehicle/Formulation: 1% carboxymethylcellulose aqueous solution in purified water

Special Features: 6 satellite females in each treated groups were added for determination of plasma levels of the test item.

No Observed Effect Level:
Fertility: greater than 2000 mg/kg/day
Maternal toxicity: 500 mg/kg/day
Embryo-fetal development: greater than 2000 mg/kg/day

Dose-level (mg/kg/day)		**0 (control)**	**500**	**2000**
Females	Toxicokinetics: AUC $_{(0\text{-}24)}$ (ng.h/ml)			
	GD 6		400978	1692913 (x4.2)
	GD 17		477387	1477919 (x3.1)
	No. evaluated (principal animals)	24	24	24
	No. Died or sacrificed moribund	0	0	2
	Clinical observations			
	- ptyalism	0	0	23
	Necropsy observations	-	-	2
	Premating body weight gain (Day 1-Day 15) (g)	31	35	33
	Gestation body weight gain (GD0-GD18) (g)	117	120	108
	Body weight at termination (GD20) (g)	391	398	383
	Carcass weight at termination (g)	317.1	318.9	302.0*
	Net weight gain (GD0-GD20) (g)	71.9	71.9	59.4**
	Premating food consumption (Day 1-Day 15) (g/day)	19	19	18
	Gestation food consumption (GD2-GD18) (g/day)	27	27	26
	Mean no. days prior to mating	2.4	2.2	3.4
	No. of females sperm-positive	24	23	22
	No. of pregnant females	24	22	21

- No noteworthy findings *: - p<0.05 ** - p<0.01 GD: gestation day ( ): ratio to lower dosage

3.3 MATING AND FERTILITY DATA (Appendices 4, 14 and 16)

The mating and fertility indexes calculated after the mating trial are summarized in the following table:

Summary of mating and fertility parameters

Dose-level (mg/kg/day)	0	500	2000
Paired females	24	24	22
Females able to mate	24[(a)]/24	23[(a)]/24	22[(a)]/22
Mating index (%)	100	96	100
Pre-coital interval (days)	2.4	2.2	3.4
Pregnant females	24[(a)]/24	22[(a)]/23	21[(a)]/22
Fertility index (%)	100	96	95
Females with live fetuses at CS	23[(b)]/23	20[(b)]/20	20[(b)]/20
Gestation index (%)	100	100	100

[(a)]: these numbers take into account the matings which were not detected (no evidence of mating but pregnant female at terminal sacrifice).
[(b)]: the live or dead status of the fetuses was not evaluated for the females with no evidence of mating.
CS: cesarean section.

All the mating, fertility and gestation parameters were similar in the control and treated groups, showing only normal fluctuation across the groups.

Maternal/Embryo-fetal findings:

Pregnancy data

Number of animals with live fetuses, dead fetuses, mean number of corpora lutea, and fetal sex ratios were not affected. Percent of pre-implantation loss was lower at both doses (500-2000 mg/kg/day), due to higher than expected values in the control group. Post-implantation losses were slightly higher at both doses (4-4.4 vs 2.0 in controls), but the difference was not statistically significant (see the table below).

Dose-level (mg/kg/day)		0 (control)	500	2000
Females				
	No. of evaluated females at hysterectomy	23	20	20
	No. aborted or with total resorption of litter	0	0	0
	Mean no. corpora lutea	16.7	16.9	16.0
	Mean no. implantations	13.2	14.8	15.1*
	% preimplantation loss	20.9	12.5**	5.9***
Litters:	No. litters evaluated	23	20	20
	No. live fetuses	297	282	290
	Mean no. resorptions + scars	0.3	0.6	0.6
	No. dead fetuses	0	0	0
	% post-implantation loss	2.0	4.4	4.0
	Mean fetal body weight (g)	3.70	3.65	3.62
	Fetal sex ratios (% males)	49.5	51.4	51.2
	Fetal external malformations	0	0	0
	Fetal soft tissue malformations	1	0	0
	Fetal skeletal malformations	2	2	0

Fetal data:

No external malformations or variations were observed. Similarly, no effects on soft tissues were observed. Fetal body weights were not affected (3.7, 3.65, and 3.62 g at 0, 500, and 2000 mg/kg/day respectively). At 2000 mg/kg/day, significant increases in incomplete ossification of supraoccipital bone were observed (litter incidences of 9, 25, and 35% respectively). At 2000 mg/kg/day, incomplete ossification of the 4th sternebra was higher in fetuses (30.7, 27.7, and 35.1% respectively) and in litters (74, 70, and 85% respectively). These were all considered to be minor abnormalities or variations. These fetal findings appear to occur at a high dose which may be associated with the maternal toxicity (decreased bodyweight gain at 2000 mg/kg/day).

Table. Fetal observations in the segment I/II study in rats

SUMMARY OF FETAL SKELETAL VARIATIONS

Dose (mg/kg/day)		0	500	2000
Litters Evaluated	N	23	20	20
Fetuses Evaluated	N	153	148	151
INCOMPLETE OSSIFICATION OF SUPRAOCCIPITAL				
Fetal Incidence	N	2 f	8	11*
	%	1.3	5.4	7.3
Litter Incidence	N	2 f	5	7
	%	8.7	25.0	35.0
Affected Fetuses/Litter	MEAN%	1.3 d	4.8	7.5
	S.D.	4.2	9.6	12.8
HEAD-OTHERS				
Litter Incidence	N	5	7	7
Fetal Incidence	N	6	10	9
UNOSSIFIED HYOID				
Fetal Incidence	N	3 f	5	1
	%	2.0	3.4	0.7
Litter Incidence	N	3 f	4	1
	%	13.0	20.0	5.0
Affected Fetuses/Litter	MEAN%	1.9 d	3.3	0.6
	S.D.	5.0	7.5	2.8
INCOMPLETE OSSIFICATION OF HYOID				
Fetal Incidence	N	3 f	5	8
	%	2.0	3.4	5.3
Litter Incidence	N	3 f	5	6
	%	13.0	25.0	30.0
Affected Fetuses/Litter	MEAN%	2.0 d	3.5	6.0
	S.D.	5.3	6.2	10.4

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test * = p<0.05

3.4.3.2 Variations

The range of skeletal variations observed was among those commonly observed in fetuses of this strain of rat, showing normal fluctuation in the degree of ossification. The fetal and litter incidences of these findings were similar in the control and the treated groups, except for a single exception, summarized in the following table:

Summary of the incidence of incomplete ossification of supraoccipital bone

Dose-level (mg/kg/day)	0	500	2000
Fetuses examined	153	148	151
Litters examined	23	20	20
Fetal incidence (%)	1.3	5.4	7.3 *
	Historical control data: 0.0 to 6.6%		
Litter incidence (%)	8.7	25.0	35.0
	Historical control data: 0.0 to 20.0%		
Affected fetuses per litter (%)	1.3	4.8	7.5
	Historical control data: 0.0 to 7.2%		

* : $p<0.05$.

Incomplete ossification of supraoccipital bone was observed at a higher incidence in the treated groups, compared to the control group. However, the differences were slight, reaching statistical significance in a single case (fetal incidence at 2000 mg/kg/day) and the values were close or within (b) historical control data. Lastly, all the other bone structures of the skull remained unaffected: interparietal, frontal and parietal bones showed normal ossification.
For all these reasons, a relationship to treatment with the test item was ruled out and the above-mentioned isolated finding was considered to be incidental.

Toxicokinetics:

Plasma levels did not accumulate over time; maximal levels were reached between 2-4 hours. Quantifiable levels were still present after 24 hours. The Sponsor stated that AUC values were similar between day 6/7 and day 17/18 and were increased in a dose proportional manner. However, the data cannot be found in appendix 20.

Rat plasma concentrations
(results as ng/ml)

Dose level (mg/kg/day)	Animal No / Time	GD 6					GD 7	GD 17					GD 18
		Predose	1h	2h	4h	8h	24h	Predose	1h	2h	4h	8h	24h
500	A20429 Female	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	A20430 Female	1279	NS	68193	NS	11780	NS	1098	NS	57451	NS	28406	NS
	A20431 Female	1657	NS	50163	NS	16342	NS	2016	NS	50683	NS	14451	NS
	A20432 Female	NS	31941	NS	49365	NS	1724	NS	52724	NS	40584	NS	1173
	A20433 Female	NS	16484	NS	52414	NS	1287	NS	38501	NS	44231	NS	1423
	A20434 Female	NS	22393	NS	34714	NS	1027	NS	39278	NS	42760	NS	1209
2000	A20459 Female	7206	NS	66096	NS	98650	NS	2661	NS	103954	NS	82216	NS
	A20460 Female	3724	NS	104110	NS	137988	NS	3825	NS	148286	NS	77798	NS
	A20461 Female	1523	NS	134668	NS	94582	NS	2080	NS	131564	NS	62476	NS
	A20462 Female	NS	39606	NS	114986	NS	2650	NS	52094	NS	143974	NS	3567
	A20463 Female	NS	37612	NS	94330	NS	5193	NS	67338	NS	115560	NS	1923
	A20464 Female	NS	49946	NS	97536	NS	3443	NS	89294	NS	139098	NS	1575

NS : no sample
GD : Gestation day

Summary of individual study findings: In a segment I/II rat fertility/embryo-fetal developmental study, carbaglu was administered orally at doses of 0, 500, and 2000 mg/kg/day to females starting at 15 days prior to mating, during mating, and up to day 17 of gestation. Females were sacrificed on day 20. Plasma concentrations of carbaglu were slightly lower at 500 mg/kg/day on GD 17 (51-57 & 104-148 µg/ml at 500 & 2000 mg/kg/day, respectively, on GD 17, compared to 50-68 & 66-135 µg/ml, respectively, on GD 6). The males were used only for mating and did not receive the drug (n=24). The drug produced maternal toxicity at the high dose (significant decreases in bodyweight gains by 17.4%, and decreases in food consumption by 4-5% on gestation days 0-20). Mean fetal weights were not altered by the drug. However, fetal toxicity was noted at 2000 mg/kg/day, where significant increases in the incidence of incomplete ossification of supraoccipital bone (fetal incidences were 9, 25, and 35% at 0, 500, and 2000 mg/kg/day, respectively), higher incomplete ossification of the 4th sternebra in fetuses (30.7, 27.7, and 35.1% respectively) and in litters (74, 70, and 85% respectively) was observed. No effects on female fertility were observed. NOAEL for maternal toxicity was 500 mg/kg/day, based on body weight decrements at 2000 mg/kg/day. Fetal NOAEL was 500 mg/kg/day, as skeletal findings were noted in fetuses at 2000 mg/kg/day due to the maternal toxicity. The Sponsor stated that the NOAEL for maternal toxicity was 500 mg/kg/day, and for fertility and embryo-fetal toxicity was 2000 mg/kg/day.

Addendum: The increases in fetal and litter incidence of incomplete ossification of 4th sternebra in the 2000 mg/kg/day group were not statistically significant. Although significant increases in incomplete ossification of the supraoccipital bone were observed (fetal incidences were 1, 5, and 7% at 0, 500, and 2000 mg/kg/day, respectively, and litter

incidences were 9, 25, and 35% at 0, 500, and 2000 mg/kg/day, respectively), the fetal and litter incidences at both doses were very close to historical control data (0 to 7% for fetal incidence, and 0 to 20% for litter incidence). No significant effects on female fertility were observed. Thus, the NOAEL for maternal toxicity is considered to be 500 mg/kg/day, based on the bodyweight gain decrement at 2000 mg/kg/day. The fetal NOAEL is considered to be 2000 mg/kg/day.

Oral (Gavage) Embryo/fetal Development study in Rabbits

Study no: 24241 RSL

Volume #, and page #: Submission 1/12/05 (# 008), volume 7/8, pg 1.

Conducting laboratory and location: (b) (4)

Date of study initiation: 4/4/2003

GLP compliance: Yes

QA report: yes (X) no ( )

Drug, lot #, radiolabel, and % purity: Batch #s 05031207P124, # 05031212P142, and 05031301P143. No purity data were provided.

Formulation/vehicle: 1% carboxy-methylcellulose aqueous solution in purified water (7.5 ml/kg/day).

Methods:
Species/strain: Rabbits, KBL New Zealand white, 20/dose + 6 satellite animals/group were used for TK.

Doses employed: 0, 250, and 1000 mg/kg/day. Dose selection was based on a previous dose-ranging study in pregnant rabbits, where doses of 500, 1000, and 2000 mg/kg/day were administered from days 6 to 18 of pregnancy. At 2000 mg/kg/day, animals were sacrificed prematurely due to severe effects on bodyweight gain and food consumption. Therefore, a high dose of 1000 mg/kg/day was selected for the current study.

Route of administration: Oral

Study design: The mated females were given carglumic acid from day 6 of gestation to day 18 of gestation and sacrificed on day 20.
Number/sex/group: 20 females/dose received carglumic acid at doses of 0, 250, and 1000 mg/kg/day. See the study design, parameters and endpoints evaluated below:

Methods

Groups of 20 mated female rabbits of the KBL New Zealand White strain received the test item, N-carbamyl-L-glutamic acid, by daily oral administration at 250 or 1000 mg/kg/day from day 6 to day 18 *post-coitum (pc)*. Six satellite animals were added to these groups for toxicokinetic purposes on days 6 and 18 *pc*.
One group of 20 females received the vehicle alone (1% carboxymethylcellulose) under the same experimental conditions and acted as a control group. A constant dose volume of 7.5 mL/kg/day was used.
During the dosing period, clinical signs were recorded daily and mortality was checked twice a day. Body weight and food consumption were recorded at designated intervals. On day 29 *pc*, the does were sacrificed and subjected to a macroscopic *post-mortem* examination. The gravid uterus was weighed to allow calculation of the net body weight gain. The fetuses were removed by hysterectomy. The following litter parameters were recorded: number of corpora lutea, implantation sites, early and late resorptions, dead and live fetuses. The fetuses were weighed and subjected to external, soft tissue and skeletal examinations. All females were submitted to a macroscopic *post-mortem* examination of the principal organs.

Results:

Mortality: One female at 1000 mg/kg/day was found dead on GD (gestation day) 20. This female had signs of maternal toxicity such as reduction or absence of food consumption from GD 6 and severe bodyweight loss (-465 g between GD 6-19). No clinical signs or necropsy findings were observed. Because a fetus was found in the bedding on the day of the death of the female, this death may be partly due to a spontaneous abortion and was not considered to be drug related.

Clinical signs: One female had blood in the bedding on GD 29; this was probably due to delivery date. Reddish contents were present in the right uterine horn.

Body weights/weight gains: Bodyweight gain was significantly decreased at 1000 mg/kg/day on GD 6 to 9 (24, 7, and -32 g at 0, 250, and 1000 mg/kg/day respectively, *$p<0.001$). Bodyweight gains were also lower on GD 6-12 and 6-15.

3.3.3 Body weight and body weight changes (Figures 1 and 2, Tables 2 to 4, Appendices 6 to 8)

The mean body weight change values are summarized in the following table:

Body weight change (g)

Dose-level (mg/kg/day)	0	250	1000
. GD 6 to 9	24	7 (-71)	-32*** (-233)
. GD 6 to 12	73	53 (-27)	4** (-94)
. GD 6 to 15	142	108 (-24)	63* (-56)
. GD 6 to 19	178	153 (-14)	80 (-55)
. GD 19 to 29	234	210 (-10)	174 (-26)
. Net weight change from GD 6	-106	-185 (75)	-186 (75)

*: p<0.05; **: p<0.01; ***: p<0.001, brackets: variation from controls in percentage.

Throughout the whole dosing period (GD 6-18), the body weight gain of treated females was lower than that of controls. It is noteworthy that during GD 6-9, this effect was more pronounced, and that females given 1000 mg/kg/day lost weight. At the end of the dosing period, body weight gain of treated females at 250 mg/kg/day was comparable to that of controls. After cessation of treatment, body weight gain of group treated at 250 mg/kg/day was similar to that of controls, whereas that of the 1000 mg/kg/day group was still lower.

Lastly, body weights of treated animals were slightly below the mean control values, but these differences never reached statistical significance.

The net body weight change calculation (reflecting the maternal body weight change independently of the uterus weight) was slightly lower in the treated groups than in the control group, but without statistical significance.

Food consumption: Food intake was significantly lower in the 1000 mg/kg/day group during GD 6 to 9 (160, 149, and 103* g/day in the 0, 250, and 1000 mg/kg/day groups, respectively).

3.3.4 Food consumption (Figure 3, Table 5, Appendix 9)

The mean variations of food consumption are summarized in the following table:

Food consumption values (g/day)

Dose-level (mg/kg/day)	0	250	1000
. GD 6 to 9	160	149 (-7)	103*** (-36)
. GD 9 to 12	163	149 (-9)	138 (-15)
. GD 12 to 15	132	112 (-15)	116 (-12)
. GD 15 to 19	159	145 (-9)	134 (-16)
. GD 19 to 29	148	134 (-9)	157 (6)

***: p<0.001 : brackets: variation from controls in percentage.

Maternal/Embryo-fetal findings:

Pregnancy data

The pregnant females delivered similar number of live fetuses (169, 204, and 151 in the 0, 250, and 1000 mg/kg/day groups, respectively). The uterine weights at 1000 mg/kg/day were lower (517, 548, and 470 g respectively). This was due to the lower number of fetuses per female at 1000 mg/kg/day (176, 208, and 151 at 0, 250, and 1000 mg/kg/day, respectively). The Sponsor explains that it was due to the slightly low number of corpora lutea and implantation sites as shown below. The number of dead fetuses/animals was actually lower at the high dose. The total number of resorptions, post-implantation losses, and mean fetal body weights were not affected. Early resorptions were slightly higher at the high dose (2, 1, and 7 in the 0, 250, and 1000 mg/kg/day groups, respectively; see the two tables below).

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HYSTERECTOMY DATA (Summary table)

Dose: (mg/kg/day)		0	250	1000
Pregnant Females Alive at Term	N	18	19	18
with Total Resorptions	N	0	0	0
with all Dead Fetuses	N	0	0	0
with Live Fetuses	N	18	19	18
Corpora Lutea	TOTAL	206	231	190
No. per animal	MEAN	11.4 d	12.2	10.6
	S.D.	2.9	2.7	2.1
Implantation Sites	TOTAL	182	214	160
No. per animal	MEAN	10.1 d	11.3	8.9
	S.D.	3.6	2.6	2.5
Preimplantation Loss	TOTAL	24 f	17	30
	%	11.7	7.4	15.8
Fetuses	N	176	208	151
No. per animal	MEAN	9.8 d	10.9	8.4
	S.D.	3.7	2.3	2.7
Alive	%	96.0	98.1	100.0
Dead	%	4.0	1.9	0.0
Live Fetuses	N	169 f	204	151
% of implantation sites		92.9	95.3	94.4
No. per animal	MEAN	9.4 d	10.7	8.4
	S.D.	3.4	2.4	2.7
Dead Fetuses	N	7 f	4	0*
% of implantation sites		3.8	1.9	0.0
No. per animal	MEAN	0.4 d	0.2	0.0
	S.D.	0.8	0.5	0.0

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test * = p<0.05

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HYSTERECTOMY DATA (*Summary table*)

Dose: (mg/kg/day)		0	250	1000
Resorptions + Scars	N	6 f	6	9
% of implantation sites		3.3	2.8	5.6
No. per animal	MEAN	0.3 d	0.3	0.5
	S.D.	0.8	0.7	1.0
Implant Scars	N	0 f	0	0
% of implantation sites		0.0	0.0	0.0
No. per animal	MEAN	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0
Resorptions: early	N	2 f	1	7
% of implantation sites		1.1	0.5	4.4
No. per animal	MEAN	0.1 d	0.1	0.4
	S.D.	0.3	0.2	1.0
Resorptions: late	N	4 f	5	2
% of implantation sites		2.2	2.3	1.3
No. per animal	MEAN	0.2 d	0.3	0.1
	S.D.	0.7	0.7	0.3
Postimplantation Loss	TOTAL	13 f	10	9
% of implantation sites		7.1	4.7	5.6
No. per animal	MEAN	0.7 d	0.5	0.5
	S.D.	1.0	0.9	1.0
Male Fetuses	N	90 f	102	79
	%	57.0	57.6	59.0
Female Fetuses	N	68 f	75	55
	%	43.0	42.4	41.0
Fetal Body Weight (g)	MEAN	38.1 d	34.9	39.9
	S.D.	6.3	3.8	6.2
Male Fetuses	MEAN	37.6 d	35.4	40.5
	S.D.	5.5	4.3	6.8
Female Fetuses	MEAN	36.7 d	34.6	39.3
	S.D.	6.6	3.8	5.7

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test

Rabbit fetal data:

At 1000 mg/kg/day, one fetus had domed head (fetus #2). This finding was associated with few malformations and variations seen at the visceral level. Fetus #2 displayed brain malformations (small size of brain and dilatation of ventricles) and a variation (presence of liquid in the cranial cavity). In fetal soft tissue examinations, incidences of distended stomach were higher in fetuses (0, 1.1, and 0.7% at 0, 250, and 1000 mg/kg/day, respectively) and litters (0, 6.3, and 6.3% respectively). Mean fetal weights were not altered with carglumic acid. No drug-related effects were noted on skeletal malformations. However, some effects on general ossification of fetuses were observed. These included incomplete ossification of frontal head skull (fetal incidences were 0.7, 1.7, and 2.2 in the 0, 250, and 1000 mg/kg/day groups, respectively; litter incidences were 6, 13, and 19 respectively), incomplete ossification of the first to 4th sternebra in fetuses/litters (3.3/25, 3.4/31, and 6/44 in the 0, 250, and 1000 mg/kg/day groups, respectively), incomplete ossification of 6th sternebra in litters (75, 88, and 88% respectively), incomplete ossification of talus in litters (6.3, 25, and 12.5% respectively),

incomplete ossification of pubis in fetuses (2, 5.7, and 5.2% respectively), and knobby ribs in litters (0, 0, and 6.3% respectively). None of the above skeletal findings were considered significant by the sponsor. (See revised summary in Addendum)

3.6.1 External observations (Tables 8 and 9, Appendix 14)

At 1000 mg/kg/day, one fetus (No. 2 from doe C30060) presented with domed head. This external malformation was associated with few malformations and variations seen at visceral observation (see below).

The number of fetuses showing external variations was 8, 1 and 0 distributed in 5, 1 and 0 litters at 0, 250 and 1000 mg/kg/day respectively. This distribution was not considered to be treatment-related.

3.6.2 Visceral observations (Tables 10 and 11, Appendix 14)

At 1000 mg/kg/day, fetus No. 2 (doe C30060) displayed brain malformations (small size of the brain and dilatation of ventricles) and variation (presence of liquid in the cranial cavity). Fetus No. 7 (female C30059) presented a malformed abdominal wall (omphalocele). This incidence observed in 2 fetuses from 2 litters of the high dose-group was lower than the one observed in the control group in which 4 fetuses from 3 different litters presented malformations and thus not indicative of any treatment-related effect.

SUMMARY OF FETAL SKELETAL MALFORMATIONS

Dose: (mg/kg/day)		0	250	1000
Litters Evaluated	N	16	16	16
Fetuses Evaluated	N	151	174	134
HEAD-SKULL				
Litter Incidence	N	1	0	0
Fetal Incidence	N	1	0	0
ACRANIA				
Fetal Incidence	N	1 f	0	0
	%	0.7	0.0	0.0
Litter Incidence	N	1 f	0	0
	%	6.3	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.6 d	0.0	0.0
	S.D.	2.3	0.0	0.0
HEAD-OTHERS				
Litter Incidence	N	1	0	0
Fetal Incidence	N	1	0	0
FUSED MAXILLA				
Fetal Incidence	N	1 f	0	0
	%	0.7	0.0	0.0
Litter Incidence	N	1 f	0	0
	%	6.3	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.6 d	0.0	0.0
	S.D.	2.3	0.0	0.0
FUSED MANDIBLE				
Fetal Incidence	N	1 f	0	0
	%	0.7	0.0	0.0
Litter Incidence	N	1 f	0	0
	%	6.3	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.6 d	0.0	0.0
	S.D.	2.3	0.0	0.0

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test

SUMMARY OF FETAL SKELETAL MALFORMATIONS

Dose: (mg/kg/day)		0	250	1000
Litters Evaluated	N	16	16	16
Fetuses Evaluated	N	151	174	134
THORACIC VERT.				
Litter Incidence	N	2	1	0
Fetal Incidence	N	2	1	0
FUSED THORACIC VERTEBRAE				
Fetal Incidence	N	1 f	0	0
	%	0.7	0.0	0.0
Litter Incidence	N	1 f	0	0
	%	6.3	0.0	0.0
Affected Fetuses/Litter	MEAN%	1.6 d	0.0	0.0
	S.D.	6.3	0.0	0.0
THORACIC VERTEBRA(E): FUSED ARCH				
Fetal Incidence	N	0 f	1	0
	%	0.0	0.6	0.0
Litter Incidence	N	0 f	1	0
	%	0.0	6.3	0.0
Affected Fetuses/Litter	MEAN%	0.0 d	0.6	0.0
	S.D.	0.0	2.3	0.0
THORACIC VERTEBRA(E): ARCH MALPOSITIONED				
Fetal Incidence	N	1 f	0	0
	%	0.7	0.0	0.0
Litter Incidence	N	1 f	0	0
	%	6.3	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.7 d	0.0	0.0
	S.D.	2.8	0.0	0.0

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test

SUMMARY OF FETAL SKELETAL MALFORMATIONS

Dose: (mg/kg/day)		0	250	1000
Litters Evaluated	N	16	16	16
Fetuses Evaluated	N	151	174	134
THORACIC VERTEBRA(E): FUSED CENTRUM				
Fetal Incidence	N	0 f	1	0
	%	0.0	0.6	0.0
Litter Incidence	N	0 f	1	0
	%	0.0	6.3	0.0
Affected Fetuses/Litter	MEAN%	0.0 d	0.6	0.0
	S.D.	0.0	2.3	0.0
STERNEBRA				
Litter Incidence	N	4	3	2
Fetal Incidence	N	5	4	2
FUSED STERNEBRAE				
Fetal Incidence	N	4 f	1	1
	%	2.6	0.6	0.7
Litter Incidence	N	3 f	1	1
	%	18.8	6.3	6.3
Affected Fetuses/Litter	MEAN%	2.6 d	0.4	0.6
	S.D.	6.2	1.8	2.3
MISALIGNED STERNEBRA(E)				
Fetal Incidence	N	0 f	2	1
	%	0.0	1.1	0.7
Litter Incidence	N	0 f	2	1
	%	0.0	12.5	6.3
Affected Fetuses/Litter	MEAN%	0.0 d	1.3	0.7
	S.D.	0.0	3.7	2.8

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test

SUMMARY OF FETAL SKELETAL MALFORMATIONS

Dose: (mg/kg/day)		0	250	1000
Litters Evaluated	N	16	16	16
Fetuses Evaluated	N	151	174	134
SPLITTED STERNEBRA(E)				
Fetal Incidence	N	0 f	1	0
	%	0.0	0.6	0.0
Litter Incidence	N	0 f	1	0
	%	0.0	6.3	0.0
Affected Fetuses/Litter	MEAN%	0.0 d	0.6	0.0
	S.D.	0.0	2.3	0.0
MISSHAPEN STERNEBRA(E)				
Fetal Incidence	N	1 f	0	0
	%	0.7	0.0	0.0
Litter Incidence	N	1 f	0	0
	%	6.3	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.4 d	0.0	0.0
	S.D.	1.7	0.0	0.0
RIB				
Litter Incidence	N	0	1	0
Fetal Incidence	N	0	1	0
FUSED RIBS				
Fetal Incidence	N	0 f	1	0
	%	0.0	0.6	0.0
Litter Incidence	N	0 f	1	0
	%	0.0	6.3	0.0
Affected Fetuses/Litter	MEAN%	0.0 d	0.6	0.0
	S.D.	0.0	2.3	0.0

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test

SUMMARY OF FETAL SKELETAL MALFORMATIONS

Dose: (mg/kg/day)		0	250	1000
TOTAL FETAL SKELETAL MALFORMATIONS				
Fetal Incidence	N	7 f	5	2
	%	4.6	2.9	1.5
Litter Incidence	N	5 f	4	2
	%	31.3	25.0	12.5
Affected Fetuses/Litter	MEAN%	5.3 d	2.9	1.3
	S.D.	10.1	5.7	3.5

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test

SUMMARY OF FETAL SKELETAL VARIATIONS

Dose: (mg/kg/day)		0	250	1000
Litters Evaluated	N	16	16	16
Fetuses Evaluated	N	151	174	134
HEAD-SKULL				
Litter Incidence	N	2	3	3
Fetal Incidence	N	2	5	3
INCOMPLETE OSSIFICATION OF FRONTAL				
Fetal Incidence	N	1 f	3	3
	%	0.7	1.7	2.2
Litter Incidence	N	1 f	2	3
	%	6.3	12.5	18.8
Affected Fetuses/Litter	MEAN%	0.6 d	1.4	2.0
	S.D.	2.3	3.9	4.2
ENLARGED FONTANEL				
Fetal Incidence	N	1 f	1	0
	%	0.7	0.6	0.0
Litter Incidence	N	1 f	1	0
	%	6.3	6.3	0.0
Affected Fetuses/Litter	MEAN%	0.4 d	0.8	0.0
	S.D.	1.7	3.1	0.0
INCOMPLETE OSSIFICATION OF PARIETAL				
Fetal Incidence	N	1 f	3	1
	%	0.7	1.7	0.7
Litter Incidence	N	1 f	2	1
	%	6.3	12.5	6.3
Affected Fetuses/Litter	MEAN%	0.6 d	1.4	0.7
	S.D.	2.3	4.1	2.8

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test

SUMMARY OF FETAL SKELETAL VARIATIONS

Dose: (mg/kg/day)		0	250	1000
Litters Evaluated	N	16	16	16
Fetuses Evaluated	N	151	174	134
INCOMPLETE OSSIFICATION OF INTERPARIETAL				
Fetal Incidence	N	1 f	0	1
	%	0.7	0.0	0.7
Litter Incidence	N	1 f	0	1
	%	6.3	0.0	6.3
Affected Fetuses/Litter	MEAN%	0.6 d	0.0	0.7
	S.D.	2.3	0.0	2.8
HEAD-OTHERS				
Litter Incidence	N	3	9	1
Fetal Incidence	N	11	20	1
INCOMPLETE OSSIFICATION OF HYOID				
Fetal Incidence	N	10 f	20	1
	%	6.6	11.5	0.7
Litter Incidence	N	3 f	9	1
	%	18.8	56.3	6.3
Affected Fetuses/Litter	MEAN%	5.4 d	11.4	0.6
	S.D.	12.0	14.3	2.5
UNOSSIFIED HYOID				
Fetal Incidence	N	1 f	0	0
	%	0.7	0.0	0.0
Litter Incidence	N	1 f	0	0
	%	6.3	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.6 d	0.0	0.0
	S.D.	2.3	0.0	0.0

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test

SUMMARY OF FETAL SKELETAL VARIATIONS

Dose: (mg/kg/day)		0	250	1000
Litters Evaluated	N	16	16	16
Fetuses Evaluated	N	151	174	134
CERVICAL VERT.				
Litter Incidence	N	1	0	0
Fetal Incidence	N	1	0	0
INCOMPLETE OSSIFICATION OF CERVICAL VERTEBRA(E)				
Fetal Incidence	N	1 f	0	0
	%	0.7	0.0	0.0
Litter Incidence	N	1 f	0	0
	%	6.3	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.8 d	0.0	0.0
	S.D.	3.1	0.0	0.0
THORACIC VERT.				
Litter Incidence	N	2	1	0
Fetal Incidence	N	2	1	0
THORACIC VERTEBRA(E) : UNOSSIFIED HEMI-CENTRUM				
Fetal Incidence	N	1 f	1	0
	%	0.7	0.6	0.0
Litter Incidence	N	1 f	1	0
	%	6.3	6.3	0.0
Affected Fetuses/Litter	MEAN%	0.7 d	0.6	0.0
	S.D.	2.8	2.3	0.0
THORACIC VERTEBRA(E) : UNOSSIFIED HEMI-VERTEBRA(E)				
Fetal Incidence	N	1 f	0	0
	%	0.7	0.0	0.0
Litter Incidence	N	1 f	0	0
	%	6.3	0.0	0.0
Affected Fetuses/Litter	MEAN%	1.6 d	0.0	0.0
	S.D.	6.3	0.0	0.0

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test

SUMMARY OF FETAL SKELETAL VARIATIONS

Dose: (mg/kg/day)		0	250	1000
Litters Evaluated	N	16	16	16
Fetuses Evaluated	N	151	174	134
THORACIC VERTEBRA(E): MALPOSITIONNED				
Fetal Incidence	N	0 f	1	0
	%	0.0	0.6	0.0
Litter Incidence	N	0 f	1	0
	%	0.0	6.3	0.0
Affected Fetuses/Litter	MEAN%	0.0 d	0.6	0.0
	S.D.	0.0	2.3	0.0
THORACIC VERTEBRA(E): INCOMPLETE OSSIFICATION OF ARCH				
Fetal Incidence	N	0 f	1	0
	%	0.0	0.6	0.0
Litter Incidence	N	0 f	1	0
	%	0.0	6.3	0.0
Affected Fetuses/Litter	MEAN%	0.0 d	0.6	0.0
	S.D.	0.0	2.3	0.0
LUMBAR VERT.				
Litter Incidence	N	2	1	0
Fetal Incidence	N	2	1	0
LUMBAR VERTEBRA(E): MALPOSITIONNED				
Fetal Incidence	N	0 f	1	0
	%	0.0	0.6	0.0
Litter Incidence	N	0 f	1	0
	%	0.0	6.3	0.0
Affected Fetuses/Litter	MEAN%	0.0 d	0.6	0.0
	S.D.	0.0	2.3	0.0

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test

SUMMARY OF FETAL SKELETAL VARIATIONS

Dose: (mg/kg/day)		0	250	1000
Litters Evaluated	N	16	16	16
Fetuses Evaluated	N	151	174	134
UNOSSIFIED LUMBAR VERTEBRA(E)				
Fetal Incidence	N	2 f	0	0
	%	1.3	0.0	0.0
Litter Incidence	N	2 f	0	0
	%	12.5	0.0	0.0
Affected Fetuses/Litter	MEAN%	2.7 d	0.0	0.0
	S.D.	8.5	0.0	0.0
STERNEBRA				
Litter Incidence	N	15	16	15
Fetal Incidence	N	121	152	108
INCOMPLETE OSSIFICATION OF 5th STERNEBRA				
Fetal Incidence	N	89 f	87	78
	%	58.9	50.0	58.2
Litter Incidence	N	14 f	15	15
	%	87.5	93.8	93.8
Affected Fetuses/Litter	MEAN%	55.2 d	51.3	52.5
	S.D.	28.2	24.8	25.0
INCOMPLETE OSSIFICATION OF 6th STERNEBRA				
Fetal Incidence	N	50 f	73	57
	%	33.1	42.0	42.5
Litter Incidence	N	12 f	14	14
	%	75.0	87.5	87.5
Affected Fetuses/Litter	MEAN%	32.8 d	43.9	41.9
	S.D.	28.6	25.5	32.0

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test

SUMMARY OF FETAL SKELETAL VARIATIONS

Dose: (mg/kg/day)		0	250	1000
Litters Evaluated	N	16	16	16
Fetuses Evaluated	N	151	174	134
UNOSSIFIED 5th STERNEBRA				
Fetal Incidence	N	16 f	47#	19
	%	10.6	27.0	14.2
Litter Incidence	N	5 f	13*	9
	%	31.3	81.3	56.3
Affected Fetuses/Litter	MEAN%	8.9 d	25.9	14.7
	S.D.	15.5	26.1	16.2
UNOSSIFIED 6th STERNEBRA				
Fetal Incidence	N	4 f	12	8
	%	2.6	6.9	6.0
Litter Incidence	N	3 f	7	5
	%	18.8	43.8	31.3
Affected Fetuses/Litter	MEAN%	2.3 d	7.1	5.4
	S.D.	5.3	10.1	9.4
INCOMPLETE OSSIFICATION OF 1st TO 4th STERNEBRA(E)				
Fetal Incidence	N	5 f	6	8
	%	3.3	3.4	6.0
Litter Incidence	N	4 f	5	7
	%	25.0	31.3	43.8
Affected Fetuses/Litter	MEAN%	2.8 d	3.6	6.1
	S.D.	5.5	5.7	7.7
ENLARGED STERNEBRA(E)				
Fetal Incidence	N	2 f	0	1
	%	1.3	0.0	0.7
Litter Incidence	N	1 f	0	1
	%	6.3	0.0	6.3
Affected Fetuses/Litter	MEAN%	1.4 d	0.0	0.6
	S.D.	5.6	0.0	2.5

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test * = $p<0.05$ # = $p<0.001$

SUMMARY OF FETAL SKELETAL VARIATIONS

Dose: (mg/kg/day)		0	250	1000
Litters Evaluated	N	16	16	16
Fetuses Evaluated	N	151	174	134
BIPARTITE OSSIFICATION OF STERNEBRA(E)				
Fetal Incidence	N	1 f	0	0
	%	0.7	0.0	0.0
Litter Incidence	N	1 f	0	0
	%	6.3	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.6 d	0.0	0.0
	S.D.	2.3	0.0	0.0
RIB				
Litter Incidence	N	16	15	16
Fetal Incidence	N	105	114	103
SHORT SUPERNUMERARY 13th RIB(S)				
Fetal Incidence	N	27 f	24	26
	%	17.9	13.8	19.4
Litter Incidence	N	10 f	11	13
	%	62.5	68.8	81.3
Affected Fetuses/Litter	MEAN%	19.0 d	13.3	19.6
	S.D.	22.0	12.3	15.7
FULL SUPERNUMERARY 13th RIB(S)				
Fetal Incidence	N	87 f	100	91
	%	57.6	57.5	67.9
Litter Incidence	N	14 f	15	16
	%	87.5	93.8	100.0
Affected Fetuses/Litter	MEAN%	59.9 d	57.6	65.7
	S.D.	35.9	30.1	20.4

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test

SUMMARY OF FETAL SKELETAL VARIATIONS

Dose: (mg/kg/day)		0	250	1000
Litters Evaluated	N	16	16	16
Fetuses Evaluated	N	151	174	134
FLOATING RIB(S)				
Fetal Incidence	N	1 f	0	0
	%	0.7	0.0	0.0
Litter Incidence	N	1 f	0	0
	%	6.3	0.0	0.0
Affected Fetuses/Litter	MEAN%	1.6 d	0.0	0.0
	S.D.	6.3	0.0	0.0
THICKENED RIB(S)				
Fetal Incidence	N	4 f	0	3
	%	2.6	0.0	2.2
Litter Incidence	N	4 f	0	3
	%	25.0	0.0	18.8
Affected Fetuses/Litter	MEAN%	6.0 d	0.0	2.2
	S.D.	13.6	0.0	5.0
KNOBBY RIB(S)				
Fetal Incidence	N	0 f	0	1
	%	0.0	0.0	0.7
Litter Incidence	N	0 f	0	1
	%	0.0	0.0	6.3
Affected Fetuses/Litter	MEAN%	0.0 d	0.0	1.0
	S.D.	0.0	0.0	4.2
METACARPAL BONE				
Litter Incidence	N	3	7	2
Fetal Incidence	N	5	18	5

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test

SUMMARY OF FETAL SKELETAL VARIATIONS

Dose: (mg/kg/day)		0	250	1000
Litters Evaluated	N	16	16	16
Fetuses Evaluated	N	151	174	134
UNOSSIFIED 1st METACARPAL(S)				
Fetal Incidence	N	5 f	18*	5
	%	3.3	10.3	3.7
Litter Incidence	N	3 f	7	2
	%	18.8	43.8	12.5
Affected Fetuses/Litter	MEAN%	2.8 d	9.6	2.9
	S.D.	7.2	13.4	9.3
HINDLIMB-OTHERS				
Litter Incidence	N	1	5	2
Fetal Incidence	N	2	6	3
INCOMPLETE OSSIFICATION OF TALUS				
Fetal Incidence	N	2 f	5	3
	%	1.3	2.9	2.2
Litter Incidence	N	1 f	4	2
	%	6.3	25.0	12.5
Affected Fetuses/Litter	MEAN%	0.8 d	2.8	1.8
	S.D.	3.3	5.6	5.2
UNOSSIFIED TALUS				
Fetal Incidence	N	0 f	1	0
	%	0.0	0.6	0.0
Litter Incidence	N	0 f	1	0
	%	0.0	6.3	0.0
Affected Fetuses/Litter	MEAN%	0.0 d	0.5	0.0
	S.D.	0.0	1.9	0.0

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test * = p<0.05

SUMMARY OF FETAL SKELETAL VARIATIONS

Dose: (mg/kg/day)		0	250	1000
Litters Evaluated	N	16	16	16
Fetuses Evaluated	N	151	174	134
PELVIS				
Litter Incidence	N	3	6	4
Fetal Incidence	N	3	10	7
INCOMPLETE OSSIFICATION OF PUBIS				
Fetal Incidence	N	3 f	10	7
	%	2.0	5.7	5.2
Litter Incidence	N	3 f	6	4
	%	18.8	37.5	25.0
Affected Fetuses/Litter	MEAN%	1.6 d	5.4	4.8
	S.D.	3.4	8.2	9.2
SPINE				
Litter Incidence	N	2	0	1
Fetal Incidence	N	2	0	1
PRESENCE OF 27 PRE-SACRAL VERTEBRAE				
Fetal Incidence	N	0 f	0	1
	%	0.0	0.0	0.7
Litter Incidence	N	0 f	0	1
	%	0.0	0.0	6.3
Affected Fetuses/Litter	MEAN%	0.0 d	0.0	0.7
	S.D.	0.0	0.0	2.8
PRESENCE OF 25 PRE-SACRAL VERTEBRAE				
Fetal Incidence	N	2 f	0	0
	%	1.3	0.0	0.0
Litter Incidence	N	2 f	0	0
	%	12.5	0.0	0.0
Affected Fetuses/Litter	MEAN%	2.7 d	0.0	0.0
	S.D.	8.5	0.0	0.0

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test

SUMMARY OF FETAL SKELETAL VARIATIONS

Dose: (mg/kg/day)		0	250	1000
TOTAL FETAL SKELETAL VARIATIONS				
Fetal Incidence	N	144 f	168	130
	%	95.4	96.6	97.0
Litter Incidence	N	16 f	16	16
	%	100.0	100.0	100.0
Affected Fetuses/Litter	MEAN%	96.0 d	97.3	94.8
	S.D.	11.5	10.7	12.8

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test

Toxicokinetics:
The C_{max} was linear with the dose and a decrease in plasma concentration was noted on GD 18 vs GD 6 (C_{max} and AUC values were at least 50% lower on GD 18 vs GD 6) suggesting modification of absorption of carglumic acid after repeated administration, as shown below.

3.7 PLASMA LEVELS OF THE TEST ITEM (Appendix 15)

Mean values (and standard deviations) for the plasma concentrations of Carbamyl-L-Glutamic acid (CGA) after single or repeated oral administrations at 250 and 1000 mg/kg/day in pregnant rabbit females are presented in the following table :

Plasma concentrations of CGA (ng/mL)

	Day 6 p.c. (1st day of treatment)		Day 18 p.c. (last day of treatment)	
Time (H)	250 mg/kg/day	1000 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
T_o	BLQ	BLQ	213 ± 64.9	183 ± 69.6
T_1	4812 ± 1405	13897 ± 2278	3651 ± 252	12202 ± 3225
T_2	7600 ± 2633	20951 ± 8641	6201 ± 3752	19903 ± 7591
T_4	12476 ± 9765	30881 ± 11362	4077 ± 458	10631 ± 4846
T_8	11195 ± 676	42154 ± 20886	5017 ± 2642	4184 ± 2267
T_{24}	1257 ± 1154	11865 ± 11567	97.3 ± 50.5	1236 ± 1634

As female C30044 (group 2) was not pregnant, plasma concentrations were not taken into account for the calculation of the group mean values.

The pharmacokinetic treatment of the data as independent models showed the results presented below :

Main pharmacokinetic parameters

	Dose-levels (mg /kg/day)			
	250	1000	250	1000
	Day *post-coitum*			
PK parameters	Day 6 p.c. (first adm.)		Day 18 p.c. (last adm.)	
AUC_{0-t} (ng.h/mL) (t=24h)	175646	654427	76238	125769
(ratio 1000/250)	3.7		1.6	
$AUC_{0-infinity}$ (ng.h/mL)	184843	804174	76716	136479
(ratio 1000/250)	4.4		1.8	
AUC extrap. (%)	4.98	18.62	0.62	7.85
C_{max} (ng/mL)	12476	42154	6201	19903
(ratio 1000/250)	3.4		3.2	
T_{max} observed (h)	4	8	2	2
(ratio 1000/250)	2.0		1.0	

Summary: In a segment II rabbit embryo-fetal developmental toxicity study of Carbaglu, oral doses of 0, 250, and 1000 mg/kg/day were administered on GD 6 through 18 (n=20). The AUC values of carglumic acid were 50% lower on day 18 (76 and 126 µg.h/ml at 250 and 1000 mg/kg/day respectively) vs on day 6 (176 and 654 µg.h/ml respectively), suggesting that carglumic acid may autoinduce its metabolism or modify its absorption. Carglumic acid produced maternal toxicity at both doses (significant decreases in bodyweight gains on GD 6-9: 24, 7, and -32 g at 0, 250, and 1000 mg/kg/day, respectively; food consumption was decreased by 7% and 35% at 500 and 1000 mg/kg/day, respectively, on GD 6 to 9). Mean fetal weights were not altered with carglumic acid. Uterine weight at 1000 mg/kg/day was 9% lower and number of fetuses/female was lower at the high dose (151 vs 176 in controls). Some effects on ossification were observed. These included incomplete ossification of frontal head skull

(fetal incidences 0.7, 1.7, and 2.2% respectively; litter incidences 6, 13, and 19 respectively), incomplete ossification of the first to 4th sternebra (fetal incidences 3.3, 3.4, and 6.0% respectively; litter incidences 25, 31.3, and 43.8%, respectively), incomplete ossification of 6th sternebra in litters (75, 88, and 88% respectively), incomplete ossification of talus in litters (6.3, 25, and 12.5% respectively), incomplete ossification of pubis in fetuses (2, 5.7, 5.2% respectively), and knobby ribs in litters (0, 0, 6.3% respectively). None of the above skeletal findings were considered significant by the sponsor. The NOAEL for maternal toxicity was <250 mg/kg/day, based on body weight decrements at 250-1000 mg/kg/day. Fetal NOAEL was also <250 mg/kg/day, as skeletal findings were noted in fetuses due to the maternal toxicity. Sponsor's NOAEL for maternal and embryo/fetal toxicity was 250 and 1000 mg/kg/day, respectively.

ADDENDUM: Upon further examination of the data being presented, the body weight and food consumption decrements at 250 mg/kg/day were not statistically significant, and the NOAEL for maternal toxicity should be considered to be 250 mg/kg/day. Also, the following effects are considered as unrelated to drug treatment due to a lack of dose dependency: the changes in uterine weights, the changes in number fetuses/female, the incomplete ossification of frontal head skull, the incomplete ossification of the first to 4th sternebra in fetuses/litters, the incomplete ossification of 6th sternebra in litters, the incomplete ossification of talus in litters, the incomplete ossification of pubis in fetuses.

Therefore, the NOAEL for maternal toxicity is considered to be 250 mg/kg/day, based on body weight decrements at 1000 mg/kg/day. Fetal NOAEL is considered to be 1000 mg/kg/day, as no significant changes were observed in any of the parameters measured.

Effects of Carbaglu on Pre- and Post-natal developmental in Rats

Study no: 24242 RSR

Volume #, and page #: Submission1/12/05 (# 008), volume 6/8, pg 1.

Conducting laboratory and location: (b) (4)

Date of study initiation: 12/23/2002

GLP compliance: Yes

QA report: yes (X) no ( )

Drug, lot #, radiolabel, and % purity: Batch #s first receipt 0503112P106, second receipt 05031207P123, and 05031207P124. No purity data were provided.

Formulation/vehicle: 1% carboxy-methylcellulose aqueous solution in purified water (10 ml/kg/day).

Methods:
Species/strain: SD rats, Crl CD SD IGS BR

Doses employed: 0, 500, and 2000 mg/kg/day. Dose selection was based on a previous segment II toxicity study in rats, where 2000 mg/kg/day did not have any effects on embryo-fetal development.

Route of administration: Oral

Study design: The mated females were given carglumic acid from day 6 of gestation to day 21 of post-partum (or until final sacrifice, whenever appropriate).

Number/sex/group: 24 females/dose received carglumic acid at doses of 0, 500, and 2000 mg/kg/day. Four satellite animals/group were similarly treated for TK analysis. The chemical analysis of carglumic acid showed that the administered doses were within 5% of acceptable range. See the study design, parameters and endpoints evaluated below:

Methods

Three groups of mated female Sprague-Dawley rats were allocated to three experimental groups and received the vehicle (carboxymethylcellulose 1%) or the test item daily by the oral route (gavage) from implantation (day 6 *post-coitum, pc*) through gestation and lactation, up to weaning of the pups (day 21 *post-partum, pp*). The treatment groups are detailed in the following table:

Group	Number of females	Dose-level (mg/kg/day)
1 (vehicle)	24 principal	0
2 (test item)	24 principal + 4 satellite	500
3 (test item)	24 principal + 4 satellite	2000

Satellite animals were treated according to the same scheme as principal animals of groups 2 and 3, but only three animals were used for determination of plasma and milk levels of the test item on day 17 pp.

In F0 parent females, clinical signs, mortality and signs of morbidity were checked twice a day, except during the acclimation period. Body weight and food consumption were recorded at designated intervals during the treatment period. All F0 females of all groups were allowed to deliver normally, and pregnancy and litter parameters were recorded.

During the lactation period, the pups were observed daily for survival and clinical signs; body weight was recorded at designated intervals. On day 4 pp, the size of each litter was adjusted to obtain eight pups per litter. Physical and reflex development was assessed by designated endpoints.

On day 22 pp, one or two males and one or two female pups per litter were selected to constitute the F1 generation, which comprised 20 males and 20 females per group.

A macroscopic *post-mortem* examination was performed on all F0 parent females and on pups which died or were not selected. Any macroscopic lesions were sampled and preserved. The F1 animals were observed daily for clinical signs and mortality. Body weight and food consumption were recorded once a week. Sexual development of both males and females was assessed. Neurobehavioral tests were conducted at designated intervals to assess auditory, visual, learning, and memory functions. After sexual maturity, F1 male and female animals were paired.

Hysterectomy of the F1 females was performed on day 15 pc and the fertility and litter data parameters were recorded.

A macroscopic *post-mortem* examination of the F1 parent animals was performed.

Animal group	Treatment	Observation/investigations
F0 females (24 females/group)	Day 6 p.c.-day 21 p.p.	Mortality, clinical signs, food consumption, body weight, parturition, gestation length, litter size, live/dead pups, necropsy, implantation sites.
Satellite animals (4 females/group 2 and 3) (plasma + milk)	Day 17 p.p.	Mortality, clinical signs, toxicokinetics
F1 progeny (F1 pups during lactation)		Mortality, body weight, clinical signs, physical development, development of reflexes, necropsy of animals not selected for F1 generation.
Selection of 20 animals/sex/group after weaning		
F1 generation (not treated)		Mortality, clinical signs, food consumption, body weight, sexual development, behavioral tests.
Mating		Mating index, pre-coital interval.
Hysterectomies (Day 15 p.c.)		Litter parameters, necropsy, fertility index

p.c.: *post-coitum;* p.p.: *post-partum*

Results:

Mortality and Clinical signs: Mortality was observed at 2000 mg/kg/day, as 2/20 pregnant females died on GD 18. Additionally, 1/18 lactating females in the 2000 mg/kg/day group died on day 17 post-partum. These deaths were drug related but the toxic mechanisms are unknown. One female had hyper-salivation from GD 12; food consumption was not affected and no necropsy findings were observed in this animal. The second female had decreased food consumption (only 2 g was consumed between GD 12-15), and the necropsy revealed presence of blackish focus in the stomach mucosa and dilation of the cecum. The death was considered to be due to regurgitation of stomach contents into lungs (reddish lungs). A large portion of carglumic acid was not absorbed in this female and remained in the intestine. The animal that died during lactation lost 10 g (between day 10-14) and had no necropsy findings.

Clinical signs: Regurgitation was observed at all doses (1/22, 1/22, and 2/22 females at 0, 500, and 2000 mg/kg/day, respectively, during pregnancy). Hyper-salivation was noted with carglumic acid (in 0/22, 1/22, and 4/20 at 0, 500, and 2000 mg/kg/day respectively).

Bodyweight: Bodyweight changes are described below. At 500 mg/kg/day, bodyweight gain was decreased transiently (until GD 9), but was more severe at 2000 mg/kg/day. Bodyweights were higher during lactation at the high dose on day 1 to 7 postpartum. Both doses decreased bodyweight gains in pups from birth to day 7 of lactation.

3.2.3 Body weight (pregnant and lactating females)
The changes in body weight over the treatment period are summarized in the table below:

Body weight changes (grams)

Dose-level (mg/kg/day)	0	500	2000
Pregnancy period	n=18	n=22	n=20 or 18
. GD 6 to 9	15	12 (-20)	7*** (-53)
. GD 9 to 12	22	23 (5)	21 (-5)
. GD 12 to 15	23	21 (-9)	25 (9)
. GD 15 to 20	68	67 (-1)	51** (-25)
. GD 6 to 20	127	123 (-3)	108** (-15)
Lactation period	n=18	n=22	n=18 or 17
. days 1 to 7 pp	25	23 (-8)	32* (28)
. days 7 to 14 pp	14	14 (0)	16 (14)
. days 14 to 21 pp	-27	-22 (-18)	-23 (-15)
. days 1 to 21	12	15 (25)	25 (108)

*: p<0.05; **: p<0.01; ***: p<0.001; in brackets: variation from controls in %

Food consumption: At 2000 mg/kg/day, food intake was decreased transiently (until GD 9). Slight reduction in food consumption occurred during lactation at both doses during day 1 to 7 and 7 to 14 postpartum.

3.2.4 Food consumption (pregnant and lactating females)
The changes in food consumption are summarized in the table below:

Food consumption (grams/day)

Dose-level (mg/kg/day)	0	500	2000
Pregnancy period	n=18	n=22	n=20 or 18
. GD 6 to 9	24	24 (0)	21** (-13)
. GD 9 to 12	26	26 (0)	25 (-4)
. GD 12 to 15	27	27 (0)	27 (0)
. GD 15 to 20	29	29 (0)	27 (-7)
Lactation period	n=18	n=22	n=18 or 17
. days 1 to 7 pp	44	40* (-9)	40 (-9)
. days 7 to 14 pp	63	59 (-6)	56** (-11)
. days 14 to 21 pp	67	64 (-4)	62 (-7)

*: p<0.05; **: p<0.01; in brackets: variation from controls in %

Gestation and delivery in F0 females: The duration of gestation was not significantly different. The gestation index was slightly lower at 1000 mg/kg/day (100, 100, and 90% at 0, 500, and 2000 mg/kg/day, respectively), and post-implantation loss was not significantly different (12.4, 8, and 7.8 % respectively). All pups were alive and did not have any external malformations.

Necropsy findings in F0 females and their pups: Dilatation of cecum was noted in 2/23 F0 females at the high dose. No significant findings were noted in their pups.

Pup survival and development during lactation: No clinical signs were observed in F1 pups. At 2000 mg/kg/day, the number of pups that died on days 1 to 4 of lactation was higher (2, 3,and 12* at 0, 500, at 2000 mg/kg/day respectively), and pups that survived the first 4 days was lower (226, 174, and 202* respectively, $p<0.001$). The number of pups surviving through 21 days was lower at the high dose (130* vs 141 in controls).

3.4 PUPS SURVIVAL AND DEVELOPMENT DURING LACTATION (Tables 9 to 11, Appendices 14 to 18)

3.4.1 Pups survival (Appendices 14 and 15)

The pup survival parameters were summarized as follows:

Pups data

Dose-level (mg/kg/day)	0	500	2000
Litters with liverborn	18	22	18
Liveborn	228	277	214
Live pups/litter (mean ± SD), day 1 pp	12.7 ± 2.9	12.6 ± 2.1	11.9 ± 3.0
Pups surviving 4 days (n)	226	274	202**
Viability index (%)	99.1	98.9	94.4
Pups dead on days 1-4 (n)	2	3	12**
Pups surviving 21 days (n)	141	176	130**
Viability index (%)	100	100	93.5

**: $p<0.01$.

There were no remarkable clinical signs during lactation period. The bodyweight data from the pups are shown below. The bodyweights of pups were slightly lower from day 1, and both bodyweight and weight gain were significantly lower from day 4 and/or 7 onwards. However, no effects were observed on physical or reflex development in pups, and sex ratio was similar.

3.4.3 Body weight (Table 10, Appendix 17)
The body weight values of pups during lactation are summarized in the following table:

Body weight and body weight change of pups (grams) during lactation

Dose-level (mg/kg/day)	0	500	2000
Pup weight			
. day 1 pn	6.9	6.4 (-7)	6.5 (-6)
. day 4 pn	10.2	9.3 (-9)	9.6 (-6)
. day 7 pn	16.8	15.1** (-10)	14.9** (-11)
. day 14 pn	35.0	32.2** (-8)	30.7*** (-12)
. day 21 pn	53.2	48.9**(-8)	46.6***(-12)
Pup weight change			
. days 1-4 pn	3.4	2.9 (-15)	3.1 (-9)
. days 4-7 pn	6.6	5.7*** (-14)	5.2***(-21)
. days 7-14 pn	18.2	17.1 (-6)	15.9*** (-13)
. days 14-21 pn	18.2	16.7 (-8)	15.9* (-13)
. days 4-21 pn	43.0	39.6* (-8)	36.9*** (-14)

*: p<0.05; **: p<0.01; ***: p<0.001; in brackets: variation from controls in %; pn: post-natal

Clinical examination of F1 animals after weaning: There were no mortalities. In the few F1 males that had clinical signs (chromodacryorrhea, area of hair loss, cutaneous lesions), none of the signs were considered significant. The mean bodyweight and weight gain from weaning to mating are shown below. The bodyweight of males was lower from day 1 to day 64, and this correlated with lower food consumption in males on days 1 to 8 (15, 14, and 13* g/animal/day at 0, 500, and 2000 mg/kg/day, respectively). Bodyweight of females was lower on day 1 at both doses, but these recovered later on days 15 to 64.

3.6.3 Body weight (Figures 7, 8, 10 and 11, Tables 17 to 20, Appendices 25 to 28)
The mean body weight and body weight gain values from weaning to mating are summarized in the following table:

Mean body weight (g) and body weight gain (g) from weaning to mating

Dose-level (mg/kg/day)	0	500	2000
Males			
Body weight			
. day 1	57	51***(-11)	48*** (-16)
. day 8	98	91* (-7)	85*** (-13)
. day 15	157	148 (-6)	140*** (-11)
. day 64	523	506 (-3)	481** (-8)
Body weight gain			
. days 1 to 8	41	41 (0)	37* (-10)
. days 8 to 15	59	57 (-3)	55 (-7)
. days 15 to 64	366	358 (-2)	341 (-7)
Females			
Body weight			
. day 1	54	50* (-7)	49** (-9)
. day 8	89	87 (-2)	83 (-7)
. day 15	133	130 (-2)	128 (-4)
. day 64	287	279 (-3)	285 (-1)
Body weight gain			
. days 1 to 8	35	37 (6)	34 (-3)
. days 8 to 15	44	43 (-2)	44 (0)
. days 15 to 64	154	149 (-3)	157 (2)

*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; in brackets: variation from controls in %

Assessment of reflex and physical development: Sponsor states that the physical developments (i.e., pinna unfolding, tooth eruption, eye opening, hair growth, and auditory canal opening) were unaffected compared to controls. Similarly, no evidence of disturbance in reflex or response (i.e., surface righting, cliff avoidance, and air righting) was observed

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F0 GENERATION

ASSESSMENT OF REFLEX AND PHYSICAL DEVELOPMENT (Mean data)

Dose (mg/kg/day)		0	500	2000
PINNA UNFOLDING day 5				
Number of pups tested	N	141	176	139
Number of pups exhibiting positive response	N	141 f	176	139
	%	100.0	100.0	100.0
HAIR GROWTH day 5				
Number of pups tested	N	141	176	139
Number of pups exhibiting positive response	N	141 f	176	139
	%	100.0	100.0	100.0
TOOTH ERUPTION day 13				
Number of pups tested	N	141	176	138
Number of pups exhibiting positive response	N	139 f	175	138
	%	98.6	99.4	100.0
EYE OPENING day 17				
Number of pups tested	N	141	176	130
Number of pups exhibiting positive response	N	141 f	176	130
	%	100.0	100.0	100.0
AUDITORY CANAL OPENING day 17				
Number of pups tested	N	141	176	130
Number of pups exhibiting positive response	N	141 f	175	130
	%	100.0	99.4	100.0
SURFACE RIGHTING day 5				
Number of pups tested	N	141	176	139
Number of pups exhibiting positive response	N	141 f	172	138
	%	100.0	97.7	99.3
CLIFF AVOIDANCE day 11				
Number of pups tested	N	141	176	138
Number of pups exhibiting positive response	N	141 f	176	138
	%	100.0	100.0	100.0
AIR RIGHTING day 17				
Number of pups tested	N	141	176	130
Number of pups exhibiting positive response	N	141 f	175	130
	%	100.0	99.4	100.0

Statistical key: f=Fishers exact test

Sexual development: The cleavage of balanopreputial gland was delayed in two males at 2000 mg/kg/day (mean age of appearance was 33, 34, 34 at 0, 500, and 2000 mg/kg/day, respectively; this was observed in 1, 1, and 2 animals respectively). However, this observation was not considered significant because the mean age values were not different from controls. In females, the mean age of vaginal opening was not affected at

500 mg/kg/day but was slightly higher at 2000 mg/kg/day (37 days as compared to 35 days in controls). Fertility was not affected.

Neurobehavioral test: Auditory function (such as acoustic startle reflex) was not affected. Similarly, visual function, learning, and memory (such as Water T-maze performance at age 6-7 weeks) were not affected.

Spontaneous locomotor activity: The mean total motor activity was higher in F1 males between the two trials (59.8, 66.3, and 75.5 over a 10-min period). However, it was not considered significant because other neurobehavioral tests were not affected.

Clinical examination of F1 animals during pregnancy: No significant effects on clinical signs or on maternal bodyweight were observed. Bodyweight and weight gains on GD 0-8 were lower in pregnant females at both doses (bodyweight gains were 47, 38, and 38 g at 0, 500, and 2000 mg/kg/day, respectively). Weight gain was reduced by 11% between GD 0-15 (89, 79, and 79 g, respectively). Food consumption was significantly lower (by up to 7%) at the high dose during pregnancy in F1 females on GD 0-8 (27, 25, and 25* g/day, respectively), correlating with lower bodyweight gains.

Mating and fertility performance: No effects on mating indices, fertility indices, gestation index, or pregnancy duration were observed. The fertility of F1 rats was not affected. No effects were observed in necropsy of F1 animals, except for a single high-dose female (1/20) with dilated renal pelvis.

Hysterectomy of F1 females on day 15 post-coitum: Implantation sites (16.1, 13.7, and 13.1* at 0, 500, and 2000 mg/kg/day, respectively) and post–implantation loss (1.3, 0.8, and 0.4*, respectively) per animal was lower. However, it was not considered significant because the number of corpora lutea and number of implantation sites were considered high in the control group.

Toxicokinetics: The plasma levels of carglumic acid did not increase in a dose proportional manner; the levels were 70 and 18 µg/ml at 500 and 2000 mg/kg/day, respectively, in F0 females. Carglumic acid was also found in the milk at both doses (12 and 36 µg/ml, respectively). The data are shown in the sponsor's table below.

3.11 PLASMA AND MILK LEVELS OF THE TEST ITEM IN THE F0 FEMALES (Appendix 40)

Plasma and milk levels of N-carbamyl-L-glutamic acid were measured on day 17 *post-partum* from satellite animals of groups 2 and 3.
Plasma samples were collected at 4 hours post dosing, which is assumed to be the approximate time where Cmax is reached in plasma (as shown in previous toxicokinetics investigations, in particular during the reproduction toxicity study conducted in pregnant female rats. (b) *Study No 23288 RSR*). Milk was collected from the same lactating dams just after the plasma samples.

Mean concentrations (±sd) of N-carbamyl-L-glutamic acid (µg/mL) in F0 parent females. (n=3 per group)

Dose-level (mg/kg/day)	Group 2 500	Group 3 2000	Ratio group 3 / group 2 x 4
Plasma	69.8 ± 28.8	17.9 ± 50.6	x 2.6
Milk	12.3 ± 9.5	36.2 ± 2.6	x 2.9
Ratio Milk/Plasma	0.16 ± 0.06	0.21 ± 0.05	

The assays of N-carbamyl-L-glutamic acid in plasma samples demonstrated that all animals were exposed to the test item. The plasma levels were consistent with the toxicokinetics data generated in pregnant rats (b) *Study No 23288 RSR*) and confirmed that the Cmax in plasma does not increase in a dose-proportional manner.
Quantifiable amounts of N-carbamyl-L-glutamic acid were also found in all milk samples demonstrating the transfer of the test item to the milk of the lactating females in a non-dose related manner. Moreover the milk / plasma levels ratios showed that the concentrations achieved in the milk were at least equivalent to 10% of the corresponding peak plasma levels. This allowed concluding that a significant percentage of N-carbamyl-L-glutamic acid was excreted in the milk of the treated lactating animals.

Summary

In the rat Segment III study (0, 500, and 2000 mg/kg/day, n=24/group), the mated females were given carglumic acid from day 6 of gestation to day 21 post-partum (or until final sacrifice). Carglumic acid at 2000 mg/kg/day produced mortalities in animals (as 2/20 pregnant females died on GD 18 and 1/18 lactating female died on day 17 postpartum). Doses of 500 and 2000 mg/kg/day produced clinical signs (hypersalivation in 0/22, 1/22, and 4/20 females respectively), decreases in bodyweight gain and food consumption (during gestation and lactation). Bodyweight was decreased in pregnant females at 2000 mg/kg/day (on GD 6-20 by up to 15%). Food intake was lower during pregnancy (GD 6-9 by 13%) and during lactation days 1-14 (by up to 11%), and during lactation days 14-21 postpartum (by up to 18%) at both doses. Pup survival was lower in the 2000 mg/kg/day group during the first 4 days (226, 274, and 202* respectively) and through age 21 days (141, 176, and 130*, respectively). Bodyweight and weight gain of pups were significantly lower from day 4 and/or day 7 of lactation (by up to 11-21%) at both doses. The bodyweight was also lower from weaning to mating in males, and was lower on day 1 in females at both doses. Physical development was not affected in F1 pups. Sexual development and neurobehavioral tests were not significantly different in drug-treated groups. In F1 females during pregnancy, bodyweight and weight gain were decreased at both doses by up to 5-6% and up to 19% respectively. Food intake was

decreased by 7% at the high dose. The number of implantation sites were reduced at 2000 mg/kg/day (16.1, 13.7, and 13.1*, respectively). Post-implantation losses were lower, (1.3, 0.8, and 0.4* number per animal respectively). The reductions in implantation sites and post-implantation losses were not considered significant.

In conclusion, the NOAEL for peri/post-natal toxicity was <500 mg/kg/day, since three F0 female rats died (two during gestation, 1 during lactation). At 2000 mg/kg/day, bodyweight and food consumption were severely reduced; these parameters were also transiently reduced at 500 mg/kg/day. However, no effects on the gestation and delivery parameters were noted in the F0 females. In the F1 pups, higher mortality rates were noted at 2000 mg/kg/day during the first 4 days of lactation, with reduced bodyweight gain in pups until the end of lactation. Carglumic acid was excreted in milk, and significant levels were present in the milk (12 and 36 µg/ml at 500 and 2000 mg/kg/day, respectively). The lower bodyweight of the F1 generation progressively returned to control values at the end of pre-mating (except in high-dose males); however, during pregnancy in F1 rats, the body weights and food consumption were lower at both doses. The AUC was not determined in this study, but plasma concentration of carglumic acid in F0 parents was 70 and 18 µg/ml at 500 and 2000 mg/kg/day (which suggests that carglumic acid may autoinduce its own metabolism). The concentration in milk was 12 and 36 µg/ml, respectively. Thus, a NOAEL could not be established in this study, and was considered <500 mg/kg/day for peri-post natal development in rats. This result may be due to maternal toxicity.

ADDENDUM:

Increased mortality in F0 females occurred at 2000 mg/kg/day, not at 1000 mg/kg/day as stated in the original review. Hyper-salivation occurred only at the high dose. Decreases in bodyweight gains, but not bodyweight, during gestation was significant only at the high dose. Decreases in food intake were transient and were not dose dependent. In F1 pups, the reduction in survival rates was noted only at the high dose. During pregnancy in F1 rats, there was a small but significant decrease in food consumption at the high dose; the small changes in body weights were not statistically significant.

2.6.6.9 Discussion and Conclusions

Single oral doses of carglumic acid up to 2800 mg/kg were generally well tolerated in rats, as no mortalities were observed, and carglumic acid did not produce any changes in body weight or necropsy findings. Thus, 2800 mg/kg is considered as a tolerated acute oral dose in rats. Similarly, in intravenous studies in rats, doses of up to 238 mg/kg were generally well tolerated, and no mortalities, changes in body weight, or necropsy findings were observed.

In the 2-week oral toxicity study of carglumic acid in neonatal rats, animals received the test article (0, 250, 500, 1000, and 2000 mg/kg/day) from day 4 to day 21 postpartum.

All doses of carglumic acid produced mortalities. The deaths were not dose dependent, and post-mortem gross pathology of the pups was consistent with gavage accidents. The deaths at the lower doses, therefore, were considered the result of gavage errors. Only deaths at 2000 mg/kg/day were considered drug related, but the direct cause of death was not determined. Nevertheless, accidental deaths were not observed in the control group. Pups at the high dose of 2000 mg/kg/day died mostly within 2-3 days of treatment. The treatment produced clinical signs in animals prior to death (coldness to touch, pallor of body extremities, emaciation, dehydration, swollen abdomen, and hypokinesia), and there was impaired weight gain. In surviving animals, the incidence of clinical signs were low (e.g., swollen abdomen, wound in urogenital area, abnormally colored feces). Carglumic acid decreased bodyweight gains at 1000 and 2000 mg/kg/day, while physical or reflex development was unaffected up to 1000 mg/kg/day. In addition, carglumic acid decreased BUN at 1000 mg/kg/day (up to 23%), and decreased urinary pH at 250 mg/kg/day (6.5 vs 7.5, no urine data were available at higher doses). At 500 and 1000 mg/kg/day carglumic acid, thymus weights were decreased (16-23%), and liver weights were increased (25-30%). Although some gross pathological findings were noted (brownish/whitish/yellowish/grayish contents in the stomach), these changes were not dose dependent and, therefore, it is concluded that there were no significant gross pathological findings at doses up to 2000 mg/kg/day. At 1000 mg/kg/day carglumic acid, the only significant histopathological finding in the main study animals was the dilated kidney pelvis in male rats (4/8 vs 1/8 in controls). Thus, the NOAEL (no observed adverse effect level) is considered to be 500 mg/kg/day, based on the reduced weight gain and the effect in kidneys at 1000 mg/kg/day.

In the 26-week oral toxicity study of carglumic acid in juvenile rats (0, 500, and 1000 mg/kg/day, 10/sex/group), there was a slight accumulation of carglumic acid with time in males, but there was a 3-fold accumulation seen in females at 500 mg/kg/day in week 26 vs week 1 (1009 vs 323 µg•h/ml). Carglumic acid produced increased salivation in the high-dose males and females. Urinary pH was generally decreased at all doses, but this effect was only significant in females at the high dose. Target organs of toxicity at the high dose were the Harderian gland (necrotizing inflammation, (M: 1/10, 1/2, 6/10; F: 5/10, no data, 9/10) and liver (multicellular hepatic necrosis, M: 0/10, 0/10, 3/10; F: 0/10, 1/10, 0/10). Kidney (interstitial mononuclear cell aggregation, M: 0/10, 4/10, 7/10; F: 1/10, 1/10, 2/10) was a target organ of toxicity at both the low and high dose. Reproductive toxicity and immunotoxicity were also evaluated in this study. The reproductive parameters, including estrous cycle and male mating and fertility parameters, were not affected. Carglumic acid did not produce immunotoxicity, as there were no significant changes in thymus and spleen weights, no effects on bone marrow cellularity in the stemum and femur, no histopathologic findings in lymphoid organs and tissue, no effects on T- or B-lymphocytes. A NOAEL was not established due to the effect in kidneys at both doses, although carglumic acid was tolerated at 500 mg/kg/day.

The genotoxicity of carglumic acid was examined in three different assays: the Ames assay, the chromosome aberration assay, and the *in vivo* micronucleus assay. Carglumic acid was not mutagenic in the Ames test. Three chromosome aberration assays in human peripheral blood lymphocytes were performed: 1) In the first assay, carglumic acid

(containing 0.3% HPA) produced, in the absence of metabolic activation, a dose related clastogenic response (at 442, 788, and 1408 µg/ml), which was statistically significant at the two higher doses. 2) In the second assay, carglumic acid containing a lower level of HPA (0.1%) was used, and the medium was neutralized. Under these conditions, carglumic acid at 333-1000 µg/ml tested negative for clastogenic activity. 3) The third assay was performed with carglumic acid (containing 0.1% HPA) to determine the effects of pH adjustment on clastogenic activity. The result was negative when the pH was adjusted (pH 7.5-7.7), but positive when pH was not adjusted (pH 7-7.4). These studies demonstrated that carglumic acid per se is not clastogenic, and that either HPA, and/or a change in pH can alter the response of the cells to carglumic acid. Thus, carglumic acid is apparently not clastogenic in the *in vitro* chromosome aberration assay in human peripheral blood lymphocytes.

Three *in vivo* micronucleus assays in rats were also conducted: 1) In the first micronucleus assay, both in the pilot study and confirmatory study, carglumic acid (containing 0.3% HPA) tested positive at 3940 and 7040 mg/kg. 2) In the second assay, two batches of carglumic acid (containing 0.3% or 0.1% HPA) were used, and both tested negative at 2000 and 7000 mg/kg/day. The reason for the negative results even with the older batch with higher HPA content is unclear. 3) In the third assay, carglumic acid at 500 and 1000 mg/kg/day (containing 0.1% HPA) was given for 4 weeks. Although the drug first tested positive at 1000 mg/kg/day in male rats, the extended analysis of the data with an increased sample size showed no significant increase in MPCE frequency at 1000 mg/kg/day. Thus, carglumic acid was negative for clastogenic activity in the 4-week *in vivo* micronucleus assay in rats.

Purified preparations of hydantoin-5-propionic acid (HPA) and diaza-cycloheptane, which are both impurities and potential metabolites of carglumic acid, were examined for their genotoxic potential. HPA was positive in the chromosomal aberration assay, but was negative in both the Ames and micronucleus assays, and diaza-cyclohepatane was negative in the Ames assay. In summary, carglumic acid appears to lack genotoxic activity, and HPA and/or a change in pH, were factors in the clastogenic effects of carglumic acid in some of the genotoxicity assays.

In the segment I/II rat fertility/embryo-fetal developmental study, carglumic acid was administered orally at doses of 0, 500, and 2000 mg/kg/day to females starting at 15 days prior to mating, during mating, and up to day 17 of gestation. The females were mated with untreated males. The pregnant females were sacrificed on day 20 of gestation. Mean fetal weights were not altered by carglumic acid. Although significant increases in incomplete ossification of the supraoccipital bone (fetal incidences were 1, 5, and 7% at 0, 500, and 2000 mg/kg/day, respectively, and litter incidences were 9, 25, and 35% at 0, 500, and 2000 mg/kg/day, respectively) were observed, the incidences at both doses were close to that of historical control data. No significant effects on female fertility were observed. Thus, the NOAEL for maternal toxicity was considered to be 500 mg/kg/day, based on the bodyweight gain decrement at 2000 mg/kg/day. The fetal NOAEL was considered to be 2000 mg/kg/day.

In the segment II rabbit embryo-fetal developmental toxicity study of carglumic acid, oral doses of 0, 250, and 1000 mg/kg/day carglumic acid were administered on GD 6 through GD 18 (n=20/group), and the animals were sacrificed on day 20 of pregnancy. Carglumic acid produced maternal toxicity (55% reduction in bodyweight gain at 1000 mg/kg/day on GD 6-19; food consumption was decreased by 35% at 1000 mg/kg/day). Mean fetal weights were not altered with carglumic acid. Uterine weights at 1000 mg/kg/day were 9% lower. Some effects on ossification were observed, however none of these skeletal findings were considered significant due to the lack of dose dependency. Therefore, the NOAEL for maternal toxicity was considered to be 250 mg/kg/day, based on body weight and food consumption decrements at 1000 mg/kg/day. The fetal NOAEL was considered to be 1000 mg/kg/day, as no significant changes were observed in any of the parameters measured.

In the Segment III study in rats, the mated females were given carglumic acid (0, 500, 2000 mg/kg/day, n=24/group) from day 6 of gestation to day 21 post-partum (or until final sacrifice). Carglumic acid produced maternal toxicity at 2000 mg/kg/day, as indicated by deaths (3/24) and impaired weight gain (-15% compared to controls) during pregnancy. During lactation, bodyweight gain was actually higher at the high dose (108% compared to controls). No effects on the gestation and delivery parameters were noted in the F0 generation.

In pups (F1 generation), slightly higher mortality rates at the high dose were noted during the first 4 days of lactation (viability index was 99.1%, 98.9%, and 94.4% at 0, 500, and 2000 mg/kg/day, respectively) and between days 4-21 post-partum (100%, 100%, and 93.5% at 0, 500, and 2000 mg/kg/day, respectively). Body weight and bodyweight gains were reduced at the low dose (-8% and -8%, respectively) and at the high dose (-12% and -14%, respectively) at the end of lactation. Carglumic acid was excreted in milk at significant levels (12 and 36 µg/ml at 500 and 2000 mg/kg/day, respectively). At weaning, body weights were lower in males (-11% and -16% at 500 and 2000 mg/kg/day, respectively) and females (-7% and -9% at 500 and 2000 mg/kg/day, respectively). The lower body weights of the F1 rats from treatment groups progressively returned to levels comparable to that of the controls at the end of the pre-mating period, except in high dose males (-8% compared to controls). Physical and sexual development were not affected in F1 animals, and results of the neurobehavioral and spontaneous locomotor activity tests were not significantly different from controls. There were no effects on mating indices, fertility indices, gestation indices, or pregnancy duration in the F1 generation. The NOAEL was considered to be < 500 mg/kg/day for peri-post natal development in rats because of the impaired bodyweight gains in pups at 500 mg/kg/day.

2.6.6.10 Tables and Figures

Not applicable

2.6.7 TOXICOLOGY TABULATED SUMMARY

Not applicable

OVERALL CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS:

The following conclusions and recommendations have been adapted, in part, from the nonclinical reviews by Dr. Indra Antonipillai under IND 61,256.

Carglumic acid (N-carbamoyl-L-glutamic acid) is a structural analog of N-acetyl-L-glutamate (NAG), which is an obligatory allosteric activator of mitochondrial carbamoyl phosphate synthetase 1 (CPS 1), the first enzyme of the urea cycle. NAG is synthesized by the enzyme N-acetylglutamate synthase (NAGS). In the absence of NAGS, NAG is not produced and plasma levels of ammonia are elevated due to impaired function of the urea cycle. NAGS deficiency is one of the most severe and rarest of the hereditary urea cycle disorders.

In vitro and *in vivo* pharmacology studies in ureotelic animals have shown evidence that carglumic acid is able to pass into the mitochondria of hepatocytes where it activates the enzyme CPS 1. Although carglumic acid was shown to be a weaker *in vitro* activator of CPS 1 as compared to the naturally occurring activator NAG, it was demonstrated *in vivo* that carglumic acid stimulates CPS 1 with greater efficiency than NAG. When injected in mice, radiolabeled N-carbamoyl-[^{14}C]L-glutamate was detected in the mitochondria, whereas N-acetyl-[^{14}C]L-glutamate was not (Rubio et al,1981, Biochemistry 20: 1969-1974). This was explained by a higher permeability of the mitochondrial membrane to carglumic acid, as compared to NAG (Meijer et al, 1982, Eur. J. Biochem. 124: 325-30), and also by a greater resistance of carglumic acid to hydrolysis by aminoacylase present in the cytosol (Kim et al, 1972, PNAS 69:3530-3). Carglumic acid (1-4 mmol/l, by IP injection) produced a 61-76% survival rate in rats following a lethal dose of ammonium acetate (10.8 mmol/l). In contrast, NAG (4 mmol/l) produced a survival rate of only 2%. Similarly, in partially hepatectomized rats, treatment with carglumic acid (1 mmol/kg) prior to the injection of ammonium acetate (3.4 mmol/l) protected rats from ammonia intoxication (ammonia levels were decreased to 225, as compared to 278 µmol/L in controls, $p<0.05$).

In the safety pharmacology studies, carglumic acid had no effects on blood pressure, heart rate, or ECG after single oral doses (250 – 1000 mg/kg/day) in instrumented conscious dogs (3M+3F). The drug did not significantly alter the cardiac action potential in isolated canine purkinje fibers up to a concentration of 10^{-4} M. Carglumic acid showed no significant effects on CNS or respiratory function in rats after single oral doses of up to 1000 mg/kg.

In pharmacokinetic studies using a single oral dose, the plasma concentration (C_{max}) of carglumic acid was 60 µg/ml in rats (at 500 mg/kg) and 74-277 µg/ml in dogs (at 1000 mg/kg). In the ADME study in dogs using an oral dose of 500 mg/kg, the T_{max} was 2.0-2.5 hr, and C_{max} was 112 µg/ml. In a multiple dose study in rats **(**26-week study), oral doses of 500 and 1000 mg/kg/day produced maximal plasma concentration (69-104 µg/ml) at 2-3 hours; no significant sex-related differences in C_{max} or AUC were noted in week 1. At week 26, there was a higher accumulation of carglumic acid in females (3-fold relative to week 1) than in males (about 2-fold relative to week 1) at 500 mg/kg/day. The difference in accumulation between males and females were smaller at 1000 mg/kg/day. The AUC at 1000 mg/kg/day was lower than at 500 mg/kg/day, which may suggest auto-induction of metabolism of carglumic acid. The inverse relationship between dose level and AUC was also observed in pregnant rabbits and rats, again suggesting auto-induction of metabolism of carglumic acid.

In a bio-disposition study of 500 mg/kg [^{14}C]CGA (carglumic acid), oral administration to male and female SD rats produced maximal radioactivity in plasma at 3 hours post-dose. The plasma concentration showed biphasic elimination, with an initial rapid elimination (70%) within 0-12 hours, followed by a slower phase over the period of 12-96 hours. The elimination continued for up to 7 days post-dose. Carglumic acid was widely distributed; cecum had the highest radioactivity, followed by small intestine membrane, kidneys, liver, mesenteric lymph nodes, cartilage, pancreas, and salivary glands. Small amounts of radioactivity were noted in the spleen, thymus, brain, and testis. Low levels of radioactivity were still noted in several tissues at 96 hours post-dose, including kidneys and liver (up to 0.025%).

Metabolism studies in rats and humans showed that after oral administration, approximately 90-95% of carglumic acid remains essentially unchanged. *In vivo* studies in rats and dogs failed to detect hydantoin-5-propionic acid, diaza-cycloheptane, or L-glutamic acid, which are all considered as potential metabolites of carglumic acid. However, CO_2 was identified as a metabolite of carglumic acid in animals and humans. In *in vitro* metabolism studies of carglumic acid in rat and human hepatocytes, no metabolites were detected. When [^{14}C]carglumic acid was administered orally (500 mg/kg) to rats, about 40% of the radioactivity was recovered in urine, 22% in feces, and 7% as expired CO_2. The elimination was rapid, as 70% of the radioactivity was eliminated within 12 hours post-dose. In dogs, after oral administration of [^{13}C]carglumic acid and [^{14}C]carglumic acid (500 mg/kg), the major route of elimination in males and females was urinary (42-50%), with 18-35% of the radioactivity eliminated in feces. The elimination was rapid, as 80% of the radioactive dose was recovered in the first 24 hours. About 1% of the orally administered dose was recovered in the expired CO_2. As seen in animals, humans metabolize a small percentage of carglumic acid to CO_2. Low levels of HPA and L-glutamic acid were present in human feces following oral administration of carglumic acid. HPA in humans is probably limited to the lumen of the digestive tract. Neither HPA nor L-glutamic acid is a significant metabolite of carglumic acid.

Results from these studies suggest that the metabolism and excretion of carglumic acid in rats and dogs differ from that observed in humans. In humans, a greater percentage of carglumic acid was eliminated in the feces (approximately 70%) compared to rats and dogs (18-35%). Also, in humans, two peaks of radioactivity were detected in plasma after oral administration of carglumic acid. The second peak, which has a T_{max} of 36-48 hours, may represent the systemic circulation of water-soluble metabolites before their pulmonary elimination. This peak was not observed in animals. Glutamic acid was identified as a metabolite of carglumic acid in human feces but not in the animal studies. Finally, HPA was detected in human feces, but not in the animal studies.

In toxicity studies, single oral doses of carglumic acid up to 2800 mg/kg were generally well tolerated in rats, as carglumic acid did not produce mortality or any changes in body weight or necropsy findings. Similarly, in intravenous studies in rats, doses of up to 238 mg/kg were generally well tolerated, and no mortalities, changes in body weight, or necropsy findings were observed.

In the 2-week oral toxicity study of carglumic acid in neonatal rats, animals were treated with 0 (vehicle), 250, 500, 1000, or 2000 mg/kg/day from days 4 to 21 postpartum. All doses of carglumic acid produced mortalities, with 100% mortality observed at 2000 mg/kg/day. The deaths in the lower dose groups were not dose dependent and post-mortem gross pathology was consistent with gavage-related injury. The deaths at the lower doses, therefore, were considered the result of gavage errors. Only deaths at 2000 mg/kg/day were considered as drug related, but the direct cause of death was not determined. Nevertheless, accidental deaths were not observed in the control group. Most deaths in the 2000 mg/kg/day group occurred within 2-3 days of dosing. The treatment produced clinical signs in animals prior to death (e.g., coldness to touch, pallor of body extremities, emaciation, dehydration, swollen abdomen, and hypokinesia), and there was a reduction in weight gain. In surviving animals, the incidences of clinical signs were low (e.g., swollen abdomen, wound in urogenital area, abnormally colored feces). Carglumic acid produced a decrease in bodyweight gains at 1000 and 2000 mg/kg/day, while physical or reflex development was unaffected up to 1000 mg/kg/day. In addition, carglumic acid produced reductions in BUN at 1000 mg/kg/day (up to 23 %), and in urine pH at 250 mg/kg/day (6.5 vs 7.5 in control group, no urine data was available at higher doses). The reduction in urine pH was likely due to the excretion of carglumic acid in urine. At 500 and 1000 mg/kg/day carglumic acid, thymus weights were decreased (16-23%), and liver weights were increased (25-30%). Although some gross pathological findings were noted (brownish/whitish/yellowish/grayish contents in the stomach), these changes were not dose dependent and, therefore, it is concluded that there were no gross pathological findings related to carglumic acid treatment. Histopathologic examination was not performed in the 2000 mg/kg/day group. At 1000 mg/kg/day, the only significant histopathologic finding was dilation of kidney pelvis in male rats (4/8, as compared to 1/8 in controls). Thus, the NOAEL (no observed adverse effect level) is considered to be 500 mg/kg/day, based on the reduced weight gain and the effect in kidneys at 1000 mg/kg/day.

A 26-week oral toxicity study of carglumic acid was performed in juvenile rats (age 28 days at the start of treatment) using dose levels of 0 (vehicle), 500, and 1000 mg/kg/day (10/sex/group). There was a high incidence of increased salivation in the high-dose group. Carglumic acid had no effects on teeth, body length, or bone mineral density. Urinary pH tended to be decreased at all doses, and this effect was significant in females at the high dose. Target organs of toxicity at 1000 mg/kg/day were the Harderian gland (necrotizing inflammation in 15/20 rats) and liver (multicellular hepatic necrosis in 3/10 males). In the 500 and 1000 mg/kg/day groups, interstitial mononuclear cell aggregation in kidney was observed (9/20 rats in each group). Reproductive toxicity and immunotoxicity were also evaluated in this study. The reproductive parameters, including estrous cycle and male mating and fertility parameters, were not affected. Carglumic acid did not produce immunotoxicity, as there were no significant changes in thymus and spleen weights, no effects on bone marrow cellularity in the stemum and femur, no histopathologic findings in lymphoid organs and tissue, no effects on T- or B-lymphocytes. A NOAEL was not established due to the effect in kidneys at both doses, although carglumic acid was tolerated at 500 mg/kg/day**.**

Carglumic acid tested negative in the Ames test. However, both positive and negative results were obtained in three separate chromosomal aberration assays in human lymphocytes. The positive result appears to be due to the impurity HPA (hydantoin-5-propionic acid). HPA itself was positive in the chromosomal aberration assay, but was negative in both the Ames test and the *in vivo* micronucleus assay. An additional factor related to the observed clastogenic activity of carglumic acid was pH. Carglumic acid containing 0.1% HPA was negative in the chromosomal aberration assay when the pH of medium was adjusted to 7.5-7.7, but was positive when no pH adjustment was made (pH 7-7.4). Both positive and negative results were obtained with carglumic acid in three separate *in vivo* micronucleus tests (one positive, two negative). One of the micronucleus tests used a 4-week treatment of rats, which yielded a negative result. The weight of evidence suggests that carglumic acid lacks genotoxic acitivity.

In the segment I/II rat fertility/embryo-fetal developmental study, carglumic acid was administered orally at doses of 0, 500, and 2000 mg/kg/day to females starting at 15 days prior to mating, during mating, and up to day 17 of gestation. The females were mated with untreated males. The pregnant females were sacrificed on day 20 of gestation. Mean fetal weights were not altered by carglumic acid. No drug-related adverse effects on embryo-fetal development occurred. No significant effects on female fertility were observed. Thus, NOAEL for maternal toxicity was considered to be 500 mg/kg/day, based on the bodyweight gain decrement at 2000 mg/kg/day. The fetal NOAEL was considered to be 2000 mg/kg/day.

In the segment II rabbit embryo-fetal developmental toxicity study of carglumic acid, oral doses of 0, 250, and 1000 mg/kg/day carglumic acid were administered on GD 6 through GD 18 (n=20), and the animals were sacrificed on day 20 of pregnancy. Carglumic acid produced maternal toxicity (55% reduction in bodyweight gain at 1000 mg/kg/day on GD 6-19; food consumption was decreased by 35% at 1000 mg/kg/day). Mean fetal weights

were not altered with carglumic acid. Uterine weights at 1000 mg/kg/day were 9% lower. No drug-related effects on embryo-fetal development were observed. The NOAEL for maternal toxicity was considered to be 250 mg/kg/day, based on body weight and food consumption decrements at 1000 mg/kg/day. The fetal NOAEL was considered to be 1000 mg/kg/day, as no significant changes were observed in any of the parameters measured.

In the Segment III peri/post natal developmental study in rats, the mated females were given carglumic acid (0, 500, or 2000 mg/kg/day, n=24/group) from day 6 of gestation to day 21 post-partum (or until final sacrifice). Carglumic acid produced maternal toxicity at 2000 mg/kg/day, as indicated by deaths (3/24) and impaired weight gain (-15% compared to controls) during pregnancy. During lactation, bodyweight gain was actually higher at the high dose (108% compared to controls). No effects on the gestation and delivery parameters were noted in the F0 generation.

In pups (F1 generation), slightly higher mortality rates at the high dose were noted during the first 4 days of lactation (viability index was 99.1%, 98.9%, and 94.4% at 0, 500, and 2000 mg/kg/day, respectively) and between days 4-21 post-partum (100%, 100%, and 93.5% at 0, 500, and 2000 mg/kg/day, respectively). Body weight and bodyweight gains were reduced at the low dose (-8% and -8%, respectively) and at the high dose (-12% and -14%, respectively) at the end of lactation. Carglumic acid was excreted in the milk at significant levels (12 and 36 µg/ml at 500 and 2000 mg/kg/day, respectively). At weaning, body weights were lower in males (-11% and -16% at 500 and 2000 mg/kg/day, respectively) and females (-7% and -9% at 500 and 2000 mg/kg/day, respectively). The lower body weights of the F1 rats from treatment groups progressively returned to levels comparable to that of the controls, except in high dose males (-8% compared to controls). Physical and sexual development was not affected in F1 animals, and results of the neurobehavioral and spontaneous locomotor activity tests were not significantly different from controls. There were no effects on mating indices, fertility indices, gestation indices, or gestation duration in the F1 generation. The NOAEL was considered to be < 500 mg/kg/day for peri-post natal development in rats because of the impaired bodyweight gains in pups at 500 mg/kg/day.

The excipients to be used in the formulation appear to be safe. FDA Inactive Ingredients Database confirms that colloidal silica, microcrystalline cellulose, hypromellose, and sodium lauryl sulfate are present in approved oral formulations at levels (e.g., mg/tablet) that exceed the estimated maximum daily dose of Carbaglu for a 60-kg patient (mg/day).

Croscarmellose sodium is contained at a level of (b) (4) per tablet. For a 60-kg patient, at the maximum daily dose (75 tablets), the daily intake of croscarmellose sodium will be (b) (4). The European Commission Scientific Committee for Food states that a level not exceeding 30 g/kg/day is acceptable. In addition, JECFA (Joint FAO/WHO Expert Committee on Food Additives) classified croscarmellose in the category of ADI (acceptable daily intake) “not specified”, a term applicable to a food substance of very low toxicity. Thus, the level of croscarmellose in the proposed formulation is acceptable.

The amount of sodium stearyl fumarate in Carbaglu tablets is (b) (4) For a 60-kg patient, at the maximum daily dose, the daily intake of sodium stearyl fumarate will be (b) (4) 21 CFR §172.826 states that this ingredient can be used as a direct food additive at an amount up to 1%, depending on the intended use. The cited regulation also states that sodium stearyl fumarate should be no less than 99% pure, and should contain not more than 0.25% sodium stearyl maleate. Thus, the level of sodium stearyl fumarate in the proposed formulation is acceptable.

(b) (4) (b) (4) is an impurity in the drug product arising from an impurity (b) (4)) in the starting material. The Sponsor's proposed limit of this impurity in commercial batches of drug substance is not more than (b) (4) The concentration of (b) (4) in the drug used in nonclinical studies was not measured, and no nonclinical studies have been conducted to examine the potential toxicity of (b) (4) Because the level of (b) (4) was undetectable in the HPLC assay with a detection limit of 0.10%, it can be concluded that this impurity in the drug product that has been administered in humans was less than 0.1%. Thus, we concur with the recommendation of Dr. Martin Haber, the Review Chemist, that the limit for (b) (4) in the drug product should be not more than (b) (4).

Hydantoin 5-propionic acid (HPA) is a degradant of carglumic acid. The proposed limit of (b) (4) in the drug product is lower than the qualification threshold (0.15%) stated in the ICH Q3B(R2) guidance for impurities in drug products. Given that HPA is negative in the Ames mutation assay and is a known endogenous metabolite of histidine, the proposed limit is acceptable.

Unresolved toxicology issues:

Toxicology studies of carglumic acid were performed only in rats. For drug development, the Agency routinely requires toxicity studies in one rodent and one nonrodent species to assure safety in clinical studies and to support market approval. These studies include histopathologic evaluation of all tissues, which allows for a detailed evaluation of drug-induced toxicity. Thus, animal toxicity studies provide safety information that cannot be acquired from clinical studies. The use of two species in toxicity testing, as compared to a single species, is expected to provide greater sensitivity for the detection of potential toxic responses in humans. Because of the proposed chronic indication of Carbaglu, chronic toxicity studies in a rodent species (6-month treatment) and a nonrodent species (9-month treatment) are needed. However, the Sponsor has not conducted any toxicity study in a nonrodent species.

In a pre-IND meeting on September 24, 2002, the Agency requested that a chronic toxicity study in a nonrodent species (in addition to a chronic rodent study) be conducted to support a NDA submission for Carbaglu. However, the Sponsor submitted toxicity studies conducted in rats only in its IND application. In a pre-NDA meeting on April 28, 2004, the Sponsor disputed the need for a chronic toxicity study in a nonrodent species, citing a "lack of toxicity observed in a relevant species" as the reason for not conducting

the study. The Sponsor also cited a lack of adverse events in humans after chronic dosing as another justification. However, histopathologic examination in nonclinical toxicity studies provides highly detailed information about potential drug-induced lesions, and such information can never be obtained from human studies. In a meeting on September 26, 2008, the FDA disagreed with the Sponsor's reasoning, and the deficiency was communicated again to the Sponsor. Adding to the importance of a nonrodent toxicity study is the fact that well-controlled clinical studies were not provided in the current NDA submission. Furthermore, the clinical safety database for long-term use of Carbaglu® is based on a very small number of NAGS patients (n=26). Therefore, toxicity evaluation in a second (nonrodent) species will provide needed information for the safety profile.

Another nonclinical issue is the absence of carcinogenicity studies. Evaluation of the carcinogenic potential of carglumic acid is needed because of the chronic indication. To this end, the Sponsor has committed to conduct a two-year carcinogenicity study in a single species following approval for marketing.

Recommendations:

1. From a nonclinical standpoint, this application should be approved.

2. The Applicant should conduct a chronic (9-month) oral toxicity study in a nonrodent species and a 2-year carcinogenicity study in a single species, as post-marketing requirements.

Suggested labeling: The labeling should be changed as described in the "EXECUTIVE SUMMARY" section of this review.

REVIEWER SIGNATURE _____

YUK-CHOW NG, PH.D.
PHARMACOLOGIST
Division of Gastroenterology Products

SUPERVISOR SIGNATURE_____CONCURRENCE YES ___ NO ___

DAVID B. JOSEPH, PH.D.
ACTING PHARMACOLOGY TEAM LEADER
DIVISION OF GASTROENTEROLOGY PRODUCTS

CC:
ORIG NDA 22-562
DGP
DGP/CSO
DGP/DR. JOSEPH
DGP/DR. NG
R/D INIT.: D. JOSEPH 11/25/09

APPENDIX/ATTACHMENTS

None

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/

YUK-CHOW NG
02/19/2010
The application is recommended for approval.

DAVID B JOSEPH
02/24/2010
I concur with Dr. Ng's recommendations. Please see my secondary review for additional comments.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 22-562 **Applicant: Orphan Europe** **Stamp Date: June 18, 2009**

Drug Name: Carbaglu **NDA/BLA Type: Original NDA**

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?		X	The Sponsor has not submitted a requested chronic (9-month) oral toxicology study in a non-rodent species.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?		X	Human dose multiples not expressed in mg/m2
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	Has the applicant addressed any abuse potential issues in the submission?			This issue will be evaluated by the CSS
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? __Yes_____

If the NDA/BLA is not fileable from the pharmacology/toxicology 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.

1. The lack of a chronic (9-month) oral toxicology study in a non-rodent species.

Yuk-Chow Ng, Ph.D. 07-21-2009

Reviewing Pharmacologist Date

David B. Joseph, Ph.D. 07-21-2009

Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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

/s/

Yuk-Chow Ng
7/21/2009 03:42:31 PM
PHARMACOLOGIST

David Joseph
7/21/2009 03:50:35 PM
PHARMACOLOGIST