CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

022416Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review #2

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 22-416
Submission date: 9/4/2012 and 2/11/2013 (resubmission)
Drug: eslicarbazepine acetate
Applicant: Sepracor
Indication: Adjunctive treatment of partial-onset seizures in adults with epilepsy

Reviewing Division: Division of Neurology Products

Introductory Comments:
In the first review cycle for this NDA, the primary pharmacology/toxicology reviewer and supervisor agreed that the nonclinical information submitted was adequate to support approval of eslicarbazepine acetate for the indication noted above. However, the pharmacology/toxicology reviewer noted a deficiency in the genotoxicity data that he concluded should be addressed by the applicant. The applicant provided information in the resubmission to address the concerns.

Genetic toxicity:
The primary concern with the previously conducted genotoxicity assays was whether the human metabolites were adequately assessed in the in vitro mammalian cell assays. In the resubmission, the sponsor provided new in vitro mammalian cell genotoxicity studies with appropriate metabolic activation to address this issue. The primary reviewer and supervisor consider these studies adequate.

Juvenile animal toxicity:
The sponsor also provided juvenile animal toxicity studies in dogs in the resubmission. Death and apparent immunotoxicity were observed at all doses in a 10-month juvenile dog study. A NOAEL was not established; consequently, the primary reviewer and supervisor recommend that an additional juvenile animal study be conducted to better characterize these effects before additional pediatric trials are conducted.

Conclusions:
As previously concluded, I agree that the nonclinical information is adequate to support approval of eslicarbazepine acetate for the proposed indication. I agree that the genotoxicity evaluation is adequate. The request for a juvenile animal study to better understand the death and immune effects observed in juvenile dogs and to potentially identify a NOAEL before conducting additional pediatric trials seems reasonable.

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/s/

PAUL C BROWN
11/07/2013

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration

Division of Neurology Products (HFD-120)
Center for Drug Evaluation and Research

Date: September 17, 2013

From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 22-416 (eslicarbazepine acetate [SEP-0002093, BIA 2-093]), Submissions SDN 56 (eCTD Sequence 0053, received 9/4/2012) and SDN 65 (eCTD Sequence 0062, received 2/11/2013)

The original NDA for eslicarbazepine acetate, received on 3/30/2009, was issued a Complete Response (CR) letter (dated 4/30/2010) due to "Significant and serious deficiencies…" None of these deficiencies involved the nonclinical data. However, in the CR letter, the sponsor was asked to address potential deficiencies in the assessment of the genotoxic potential of eslicarbazepine. The in vitro (chromosomal aberration, mouse lymphoma *tk*) assays in mammalian cells were conducted using rat liver S9 for metabolic activation. Since the metabolic profile of eslicarbazepine is markedly different between rat and human, these assays were considered inadequate.

The sponsor responded to the CR letter in Submissions SDN 56 and 65. Submission SDN 56 was considered an incomplete response (Acknowledge Incomplete Response letter [AIR], dated 11/2/2012), but it did provide the following nonclinical studies:

- Document 093-884: in vitro chromosomal aberration assay in human peripheral blood lymphocytes (eslicarbazepine [(S)-licarbazepine, BIA 2-194])
- Document 0093-885: in vitro bacterial reverse mutation (Ames) assay (BIA 2-093)
- Document 093-892: in vitro bacterial reverse mutation (Ames) assay (BIA 2-093, BIA 2-194, (R)-licarbazepine [BIA 2-195], oxcarbazepine)
- Document 093-800: 28-day dose-ranging study in juvenile Beagle dog (BIA 2-093)
- Document 093-866: 10-month oral juvenile animal study in Beagle dog (BIA 2-093)
- Document 093-886: 21-day dose-ranging study in CD-1 mouse (BIA 2-093)

A number of primary and secondary pharmacology studies were also submitted, as well as the following safety pharmacology studies:

- Document 093-464: Irwin test in NMRI mouse (BIA 2-093, BIA 2-194)
- Document 093-465: Rotarod test in NMRI mouse (BIA 2-093, BIA 2-194)
- Document 093-466: Irwin test in NMRI mouse (carbamazepine, oxcarbazepine)
- Document 093-467: Rotarod test in NMRI mouse (carbamazepine, oxcarbazepine)
- Document 096-468: Modified rotarod test in MNRI mouse (BIA 2-093, BIA 2-194)

[All nonclinical studies relevant to assessment of abuse/dependency potential submitted to the NDA were consulted to the Controlled Substance Staff.]

The sponsor responded to the AIR letter in Submission SDN 65. No new nonclinical studies were provided in that submission.

The nonclinical studies submitted in the original NDA submission have been previously reviewed (*cf. Pharmacology/Toxicology NDA Review and Evaluation, NDA 22416, Christopher D. Toscano, PhD, 4/14/2010; Memorandum, NDA 22-416, Lois M. Freed, PhD, 4/28/2010; Tertiary Pharmacology Review, NDA 22-416, Paul C. Brown, PhD, 4/29/2010*). No approval issues were identified at that time. The nonclinical studies submitted in SDN 56 (with the exception noted above) have been reviewed in detail by Dr. Toscano (*Pharmacology/Toxicology NDA Review and Evaluation, NDA 22-416, Christopher D. Toscano, PhD, 9/6/2013*). Based on his review, Dr. Toscano has concluded that the nonclinical studies submitted to NDA 22-416 support approval of the NDA, with no postmarketing requirements to support use in adults. However, based on his review of the pivotal juvenile animal toxicology study, Dr. Toscano has concluded that additional nonclinical data would be needed to support use in pediatric patients (<12 years of age). In the 10-month toxicity study in juvenile dog, deaths (spontaneous deaths and moribund sacrifices) occurred at all doses; in the majority of premature deaths, there was evidence of lymphoid depletion in lymphoid tissues and bone marrow hypocellularity. Therefore, a NOAEL was not identified for either death or potential immunosuppressive effects. As Dr. Toscano noted in his review, similar findings were not observed in oral toxicity studies in adult dog (of up to 12 months in duration) at similar plasma exposures.

Conclusions/Recommendations

The sponsor has provided adequate nonclinical data to support approval for eslicarbazepine acetate for treatment of adult patients with partial-onset seizures.

The sponsor has not adequately characterized the toxicity of eslicarbazepine acetate in juvenile animals. Based on the data submitted, it appears that juvenile animals have greater or unique sensitivity to eslicarbazepine, as evidenced by convulsions and adverse effects on bone and lymphoid tissue observed only in juvenile animals. Adverse effects

on lymphoid tissue (including hypocellularity of bone marrow) were observed at all doses in juvenile animals. The relationship between the effects on lymphoid tissue and deaths, which also occurred at all doses, is unclear. Although lymphoid tissue findings were not observed in recovery animals, this cannot be taken as evidence of reversibility since main-study survivors were not affected. These findings occurred in juvenile animals at plasma exposures (C_{max}, AUC) within the range of those in adult epilepsy patients at the proposed therapeutic doses. Unless efficacious plasma exposures are substantially lower in the pediatric population compared to adults, the findings are considered clinically relevant to pediatric patients. However, certain aspects of the pivotal study (e.g., lower body weights in affected animals) complicate the interpretation of the study data. Additional data in juvenile animals would inform decisions regarding safe starting doses and the need for enhanced monitoring of pediatric patients during clinical development. Therefore, to support clinical trials in pediatric patients <12 years of age, the sponsor should conduct a study in juvenile dog, focused on further characterizing the potential immunotoxicity of eslicarbazepine acetate. This study should be conducted as a Postmarketing Requirement, if pediatric clinical trials are to be required under PREA.

Labeling recommendations: A detailed description of nonclinical findings relevant for labeling may be found in Dr. Toscano's reviews. The following labeling recommendations take into account those provided by Dr. Toscano in his reviews of 4/14/2010 and 9/6/2013. Comments on selected portions of labeling follow.

- Established Pharmacologic Class (**Highlights**) and **Section 12.1**

The sponsor does not propose an EPC for eslicarbazepine acetate; however, the sponsor's pharmacology data and published literature (e.g., Ambrosio AF *et al. Neurochem Res* 27(1/2):121-130, 2002; Benes J *et al. J Med Chem* 42:2582-2587, 1999; McCormack PL, Robinson DM *CNS Drugs* 23(1):71-79, 2009; Singh RP, Asconape JJ *J CNS Dis* 2:179-187, 2011; Zaccara G *et al*. *Seizure* 22:528-536, 2013) for eslicarbazepine suggest that the primary mechanism of action is through inhibition of voltage-gated sodium channels. However, "voltage-gated sodium channel" (VGSC) blocker or inhibitor is not an EPC. The EPC for other VGSC blockers (e.g., lamotrigine, lacosamide, topiramate, carbamazepine) is "antiepileptic drug."

(b) (4)

Considering the lack of a clear understanding of the mechanism by which eslicarbazepine acetate exerts its therapeutic effect in patients with partial-onset seizures, it seems that the best approach would be to omit an EPC term from labeling. The EPC used for approved drugs with similar pharmacology ("antiepileptic drug") provides no useful information for a drug approved to treat seizures in patients with epilepsy.

- **Section 8: Use in Specific Populations**

8.3 Nursing Mothers

The sponsor provided a published case report to support the statement that eslicarbazepine is excreted in human milk (Bulau P *et al. Eur J Clin Pharmacol* 34:311-313, 1988). However, the mother was being treated for complex partial seizures with oxcarbazepine (oral), not eslicarbazepine acetate, throughout pregnancy and continuing during lactation. Oxcarbazepine (OXC) and 10-OH-CB (10-hydroxy-carbazepine; S/R-licarbazepine) was detected in newborn plasma during the first 5 days postpartum. At birth, plasma levels of OXC and 10-OH-CB were similar in the mother and newborn; by postnatal day 5, plasma levels were only 12 and 7%, respectively, of those on postnatal day 1. The time(s) of milk sampling was not specified and the authors noted that OXC and 10-OH-CB could not be precisely measured because "The high lipid content of breast milk interfered with the GC/MS determination…" Drug levels in milk were not specified; the authors stated that the concentration ratios of milk-to-plasma were "about 0.5" for both compounds. In neither maternal or newborn biological samples were drug levels quantitated using a chiral assay; therefore, presence of eslicarbazepine in human milk or in newborn plasma cannot be confirmed.

The sponsor conducted a single oral dose (150 mg/kg) study of radiolabeled eslicarbazepine acetate in lactating CD-1 mice. Dams delivered "approximately 4 days prior to dosing." Maternal plasma and milk samples were not collected for determination of radioactivity. Levels of radioactivity in pup carcasses at 1, 4, 12, and 24 hrs post dose were 239, 3221, 3566, and 2170 ng-equiv/g, respectively. The increase in carcass radioactivity from 1 to 12 hrs post dose suggests maternal transfer of drug-related material via the milk. However, considering the possible sources of contamination and without more direct documentation of excretion into milk, it seems premature to add this information to labeling.

8.4 Pediatric Use

- **Section 13: Nonclinical Toxicology**

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Mutagenesis

In general, the results of the genetic toxicology assays were clear. However, eslicarbazepine acetate was characterized as "weakly mutagenic" or "very weakly mutagenic" in the in vitro mouse lymphoma *tk* assay, in the presence and absence of metabolic activation (rat S9). The positive finding was reproducible (two assays for each condition) in the absence, but not presence, of rat S9. Characterization of eslicarbazepine acetate as weakly or very weakly mutagenic in the in vitro mouse lymphoma *tk* assay was

based on the fact that it was negative using the Global Evaluation Factor method but positive (as indicated above) by trend test.

According to ICH S2(R1), positive findings should not be considered biologically relevant if they are (1) weak/equivocal and not reproducible or (2) small, statistically significant increases but "within the confidence intervals of the appropriate historical control values for the testing facility." For eslicarbazepine acetate, the biological relevance of the positive findings in the presence of S9 may be dismissed based on lack of reproducibility; however, the biological relevance of the positive findings in the absence of S9 cannot be dismissed based on either of these criterion. Since descriptions such as "weak" or "equivocal" should be avoided in labeling, the findings in the in vitro mouse lymphoma *tk* assay should be described as positive in the absence of S9.

Findings in the presence of rat S9 may not be of particular relevance to humans since the in vitro and in vivo metabolic profiles for eslicarbazepine acetate in rat are markedly different compared to human. However, it is not possible to determine what compound(s) are responsible for positive findings in the presence of rat S9. In addition, neither in vitro assay in which eslicarbazepine acetate was positive (in vitro chromosomal aberration assay in CHO cells, in vitro mouse lymphoma *tk* assay) was repeated using human S9. Therefore, positive findings in the presence of rat S9 should be described in labeling.

SPONSOR (7/24/2013)	RECOMMENDED
HIGHLIGHTS OF PRESCRIBING INFORMATION	
-----INDICATIONS AND USAGE-----	**-----INDICATIONS AND USAGE-----**
TRADENAME is indicated for adjunctive therapy in the treatment of partial-onset seizures in patients 18 years and older (1.1)	TRADENAME is indicated for adjunctive therapy in the treatment of partial-onset seizures in patients 18 years and older (1.1)
-----USE IN SPECIFIC POPULATIONS-----	**----USE IN SPECIFIC POPULATIONS-----**
• (b) (4)	• Pregnancy: based on animal data, may cause fetal harm. (8.1)
FULL PRESCRIBING INFORMATION	
8 USE IN SPECIFIC POPULATIONS	**8 USE IN SPECIFIC POPULATIONS**
8.1 Pregnancy Pregnancy Category C (b) (4)	**8.1 Pregnancy** Pregnancy Category C There are no adequate and well-controlled studies in pregnant women. In oral studies conducted in pregnant mice and rabbits, eslicarbazepine acetate demonstrated developmental toxicity, including teratogenicity (mice) and fetal growth retardation, at clinically relevant doses. TRADENAME should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. When eslicarbazepine acetate was orally administered (150, 350, 650 mg/kg/day) to pregnant mice throughout organogenesis, increased incidences of fetal malformations was observed at all doses and fetal growth retardation was observed at the mid and high doses. A no-effect dose for adverse developmental effects was not identified. At the lowest dose tested, plasma eslicarbazepine exposure (C_{max}, AUC) is less than that in humans at the maximum recommended human dose (MRHD) of 1200 mg/day. Oral administration of eslicarbazepine acetate (40, 160, 320 mg/kg/day) to pregnant rabbits throughout organogenesis resulted in fetal growth retardation and increased incidences of skeletal variations at the mid and high doses. The no-effect dose (40 mg/kg/day) is less than the MRHD on a mg/m^2 basis. Oral administration to pregnant rats (65, 125, 250 mg/kg/day) throughout organogenesis resulted in embryolethality at all doses, increased incidences of skeletal variations at the mid and high doses, and fetal growth retardation at the high dose. The lowest dose tested (65 mg/kg/day) is less than the MRHD on a mg/m^2 basis.

(b) (4)	When eslicarbazepine acetate was orally administered to female mice during pregnancy and lactation (150, 350, 650 mg/kg/day), the gestation period was prolonged at the highest dose tested. In offspring, a persistent reduction in offspring body weight and delayed physical development and sexual maturation were observed and the mid and high doses. The lowest dose tested (65 mg/kg/day) is less than the MRHD on a mg/m^2 basis. When eslicarbazepine acetate was orally administered (65, 125, 250 mg/kg/day) to rats during pregnancy and lactation, reduced offspring body weight was seen at the mid and high doses. Delayed sexual maturation and a neurological deficit (decreased motor coordination) were observed at the highest dose tested. The no-effect dose for adverse developmental effects (65 mg/kg/day) is less than the MRHD on a mg/m^2 basis. The data in rat are of limited relevance to human because of a marked difference in metabolic profile between species. **Pregnancy Registry** To collect information on the effects of in utero exposure to TRADENAME, physicians should encourage pregnant patients taking TRADENAME to enroll in the NAAED Pregnancy Registry. This can be done by calling 1-888-233-2334 (toll-free), and must be done by the patients themselves. Information on the registry can be found at the website, http://www.aedpregnancyregistry.org/.
(b) (4)	*This section should be omitted.*
8.3 Nursing Mothers (b) (4)	**8.3 Nursing Mothers** It is not known whether this drug is excreted in human milk. Because of the potential for serious adverse reactions in nursing infant from TRADENAME, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.
8.4 Pediatric Use (b) (4)	**8.4 Pediatric Use** Safety and effectiveness in pediatric patients have not been established. In a juvenile animal study in which eslicarbazepine acetate (40, 60, 160 mg/kg/day) was orally administered to young dogs for 10 months starting on postnatal day 21, mortality

(b) (4)	and evidence of immunotoxicity (bone marrow hypocellularity and lymphoid tissue depletion) were observed at all doses. Convulsions were seen at the highest dose tested. Adverse effects on bone growth (decreased bone mineral content and density) were seen in females at all doses at the end of the dosing period, but not at the end of a 2-month recovery period. None of these findings were reported in adult dogs dosed with eslicarbazepine acetate for up to 12 months in duration. A no-effect dose for adverse effects on juvenile dogs was not identified.
12 CLINICAL PHARMACOLOGY	
12.1 Mechanism of Action (b) (4)	**12.1 Mechanism of Action** TRADENAME is extensively converted to eslicarbazepine, which is considered to be responsible for therapeutic effects in humans. The precise mechanism(s) by which eslicarbazepine exerts anticonvulsant activity is unknown but is thought to involved inhibition of voltage-gated sodium channels.
13 NONCLINICAL TOXICOLOGY	
13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility (b) (4)	**13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility** **Carcinogenesis** In a two-year carcinogenicity study in mice, eslicarbazepine acetate was administered orally at doses of 100, 250, and 600 mg/kg/day. An increase in the incidence of hepatocellular adenomas and carcinomas was seen at 250 and

(b) (4)	600 mg/kg/day in males and at 600 mg/kg/day in females. The dose not associated with an increase in tumors (100 mg/kg/day) is less than the maximum recommended human dose of 1200 mg/day on a mg/m^2 basis. **Mutagenesis** Eslicarbazepine acetate and eslicarbazepine were not mutagenic in the in vitro Ames assay. In in vitro assays in mammalian cells, eslicarbazepine acetate and eslicarbazepine were not clastogenic in human peripheral blood lymphocytes; however, eslicarbazepine acetate was clastogenic in Chinese hamster ovary (CHO) cells, with and without metabolic activation. Eslicarbazepine acetate was positive in the in vitro mouse lymphoma *tk* assay in the absence of metabolic activation. Eslicarbazepine acetate was not clastogenic in the in vivo mouse micronucleus assay. **Impairment of Fertility** When eslicarbazepine acetate (150, 350, and 650 mg/kg/day) was orally administered to male and female mice prior to and throughout the mating period, and continuing in females to gestation day 6, there was an increase in embryolethality at all doses. The lowest dose tested is less than the maximum recommended human dose (1200 mg/day) on a mg/m^2 basis. When eslicarbazepine acetate (65, 125, 250 mg/kg/day) was orally administered to male and female rats prior to and throughout the mating period, and continuing in females to implantation, lengthening of the estrus cycle was observed at the highest dose tested. The data in rat are of limited relevance to human because of a marked difference in metabolic profile between species.

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/s/

LOIS M FREED
09/17/2013

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	NDA 22-416
Supporting document/s:	56, 65
Applicant's letter date:	8/31/2012; 2/10/2013
CDER stamp date:	9/4/2012; 2/11/2013
Product:	Eslicarbazepine acetate
Indication:	Adjunctive therapy in the treatment of partial-onset seizures in patients with epilepsy 18 years and older
Applicant:	Sunovion Pharmaceuticals, Inc.; Marlborough, MA
Review Division:	Division of Neurology Products (DNP)
Reviewer:	Christopher D. Toscano, Ph.D., DABT
Supervisor:	Lois M. Freed, Ph.D.
Division Director (acting):	Eric Bastings, M.D.
Project Manager:	Su-Lin Sun, Pharm. D.

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1 Executive Summary

1.1 Introduction

This review represents the nonclinical evaluation of the sponsor's resubmission of NDA 22-416 (dated 9/4/2012 and 2/11/2013); these submissions were in response to the Complete Response (CR) Letter dated 4/30/10. In the CR letter, the sponsor was reminded of the need to document the adequacy of the *in vitro* genotoxicity assays provided in the initial submission of this NDA. Specifically, the Division stated that due to the fact that the metabolic activation system (liver S9) was from rat, the sponsor should either demonstrate that the completed genotoxicity assays are adequate or conduct additional assessment by using an appropriate metabolic activating system. Rat is not an appropriate animal model for nonclinical testing of eslicarbazepine acetate due to its propensity to form oxcarbazepine (OXC) as the main metabolite of eslicarbazepine acetate; OXC is a minor metabolite of eslicarbazepine acetate in humans. With the genetic toxicology studies contained in the recent submission, the sponsor has fulfilled the nonclinical request made in the CR letter by performing adequate assessments of the genotoxicity of eslicarbazepine in the presence and absence of human hepatic S9. The sponsor has also submitted the results of additional primary pharmacology and CNS safety pharmacology studies. Although not necessary for approval of the current NDA, the sponsor has also provided the results of toxicology studies conducted in juvenile beagle dogs. These studies are reviewed below and labeling recommendations based on the results of these studies are provided.

1.2 Brief Discussion of Nonclinical Findings

A full review of the nonclinical studies provided in the initial submission of NDA 22-416 can be found in the nonclinical review filed in DARRTS on 4/14/2010. The results of the primary pharmacology, CNS safety pharmacology, genetic toxicology, and juvenile toxicology studies provided in the 9/4/2012 and 2/11/2013 submission are briefly discussed below.

In vitro receptor binding studies confirm that eslicarbazepine is an antagonist of several isoforms of the voltage-gated sodium channel (VGSC) and the T-type voltage-gated calcium channel ($Ca_V3.2$). CNS safety pharmacology assessment using the Irwin test and Rotarod test were performed in mice dosed with eslicarbazepine acetate (BIA 2-093) and, for comparison, in mice dosed with eslicarbazepine (BIA 2-194), carbamazepine (CBZ), and OXC. Similar clinical signs and test article-related observations occurred in the Irwin test in mice dosed with BIA 2-093, BIA 2-194, CBZ, and OXC.

BIA 2-194 was negative in the presence or absence of human S9 fraction in the *in vitro* bacterial reverse mutation assay and the *in vitro* chromosomal aberration assay in human peripheral lymphocytes.

The sponsor conducted a toxicology assessment of BIA 2-093 in juvenile beagle dogs. While not necessary for approval, since the sponsor is not seeking a pediatric indication, the results of this assessment should be described in labeling. Death (either found dead or euthanasia *in extremis*) occurred at all dose levels (40, 80, 160 mg/kg) in the pivotal study. Bone marrow and lymphoid tissue depletion was observed only in the early decedents. Convulsions were observed in juvenile animals dosed with 160 mg/kg eslicarbazepine acetate. Although the sponsor did not explicitly report convulsions in adult animals dosed with 160 mg/kg BIA 2-093, it should be noted that adult animals

dosed at this level did exhibit, according to the sponsor, “striking, seizure-like clinical signs.”

1.3 Recommendations

1.3.1 Approvability: The sponsor has fulfilled the nonclinical request made in the CR letter for adequate evaluation of the genotoxicity of eslicarbazepine; therefore, there are no outstanding nonclinical issues. The current submission, along with the initial NDA submission, contains adequate information to perform a complete nonclinical evaluation of the safety of eslicarbazepine acetate. The nonclinical information provided to date is sufficient to support the approval of NDA 22416.

1.3.2 Additional Non Clinical Recommendations: Given the inability of the sponsor to identify a NOAEL in the pivotal juvenile toxicology study conducted in dogs, it is not possible to determine the safety of eslicarbazepine acetate in a pediatric population. Additional nonclinical studies conducted in juvenile animals will be required to make a determination on the safe use of eslicarbazepine acetate in a pediatric population.

1.3.3 Labeling: Proposed nonclinical labeling for Highlights of Prescribing Information and Sections 8, 12.1, and 13 can be found in the previous nonclinical review (Toscano CD, 4/14/2010). The sponsor’s and reviewer’s proposed labeling for Section 8.4 Pediatric Use are provided below.

Sponsor’s Proposed Labeling	**Reviewer’s Proposed Labeling**
8.4 Pediatric Use (b) (4)	**8.4 Pediatric Use** Juvenile Animal Data Oral administration of eslicarbazepine acetate (40, 80, 160 mg/kg/day) to young beagle dogs for 10 months, starting on postnatal day 21, resulted in death at all dose levels; bone marrow and lymphoid tissue depletion was observed in a majority of the early decedents. CNS signs were common at > 40 mg/kg/day and included impaired coordination, tremors, labored respiration, rigidity, decreased activity, impaired equilibrium, and nystagmus. Convulsion occurred in juvenile animals, dosed with 160 mg/kg eslicarbazepine acetate. Bone mineral content, bone area, and bone mineral density were decreased in all female dose groups at the end of the dosing period but not at the end of a 2-month recovery period. A dose at which there was no observed adverse effect was not identified in this study.

2 Drug Information: Refer to the previous nonclinical review of NDA 22416 filed in DARRTS on 4/14/2010.

3 Studies Submitted

3.1 Studies Reviewed: See sponsor's tables below.

Pharmacology

Overview	Test Article: SEP-0002093, Eslicarbazepine, (R)-Licarbazepine, (b) (4) Oxcarbazepine, and Carbamazepine			(b) (4)
Type of Study	**Test System[a]**	**Method of Administration**	**Testing Facility**	**Study No. (Document No.)**
Primary Pharmacodynamics [b]				
Effect of BIA 2-194 and BIA 2-195 on hNa$_V$1.1 Peak Currents Recorded from Transfected CHO Cells – IC$_{50}$ Determination	Human Na$_V$1.1 Channels in CHO Cells	*In vitro*	(b) (4)	99942 (093-435)
Effect of BIA 2-194 and BIA 2-195 on Na$_V$1.2 Peak Currents Recorded from Transfected CHO Cells – IC$_{50}$ Determination	Rat Na$_V$1.2 Channels in CHO Cells	*In vitro*		99943 (093-436)
BIA2-194, BIA2-195, Oxcarbazepine, and Carbamazepine: Effect on Rat Na$_V$1.2 Peak Currents Recorded from Transiently Transfected CHO Cells at -70 mV – IC$_{50}$ Determination	Rat Na$_V$1.2 Channels in CHO Cells	*In vitro*		A0184 (093-470)
Oxcarbazepine: Effect on Rat Na$_V$1.2 Peak Currents Recorded from Transiently Transfected CHO Cells at -80 mV – IC$_{50}$ Determination	Rat Na$_V$1.2 Channels in CHO Cells	*In vitro*		A0185 (093-473)
Effect of BIA 2-194 and BIA 2-195 on hNa$_V$1.3 Peak Currents Recorded from Transfected CHO Cells – IC$_{50}$ Determination	Human Na$_V$1.3 Channels in CHO Cells	*In vitro*		99944 (093-437)
BIA 2-194, BIA 2-195, Oxcarbazepine and Carbamazepine: Effect on Human Na$_V$1.3 Peak Currents Recorded from Transiently Transfected CHO Cells at -60 mV – IC$_{50}$ Determination	Human Na$_V$1.3 Channels in CHO Cells	*In vitro*		A0183 (093-472)

Overview	Test Article: SEP-0002093, Eslicarbazepine, (R)-Licarbazepine, (b) (4) Oxcarbazepine, and Carbamazepine			(b) (4)
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study No. (Document No.)**
Effect of BIA 2-194 and BIA 2-195 on hNa$_V$1.7 Peak Currents Recorded from Transfected CHO Cells – IC$_{50}$ Determination	Human Na$_V$1.7 Channels in CHO Cells	*In vitro*	(b) (4)	A0031 (093-438)
BIA 2-194, BIA 2-195, and Oxcarbazepine: Effect on Ca$_V$3.2 (T-Type) Channels Recorded from Stably Transfected HEK293 Cells	Human Ca$_V$3.2 Channels in HEK293 Cells	*In vitro*		99960 (093-439)
BIA 2-194, BIA 2-195, and Oxcarbazepine: Effect on Ca$_V$2.1 Channels Recorded from Transiently Transfected CHO Cells	Human Ca$_V$2.1 Channels in CHO Cells	*In vitro*		99965 (093-440)
BIA 2-194 and BIA 2-195: Effects on the Endogenous Potassium Channels Recorded from N1E-115 Mouse Neuroblastoma Cells – IC$_{50}$ Determination	N1E-115 Neuroblastoma Cells	*In vitro*		A0195 (093-471)
Effect of BIA 2-194 and BIA 2-195 on K$_V$7.2 Currents Recorded from Transiently Transfected CHO Cells – IC$_{50}$ Determination	Human K$_V$7.2 Channels in CHO Cells	*In vitro*		99925 (093-441)
BIA 2-194 and BIA 2-195: Modulatory Effects on Recombinant AMPA Channel Currents Recorded from Transiently Transfected CHO Cells	Human AMPA Channels in CHO Cells	*In vitro*		99929 (093-442)
BIA 2-194 and BIA 2-195: Modulatory Effects on Recombinant NMDA Channel Currents Recorded from Transiently Transfected HEK Cells	Human NMDA Channels in HEK293 Cells	*In vitro*		99931 (093-444)
Effect of BIA 2-194 and BIA 2-195 on CIC-2 Currents Recorded from Transfected CHO Cells – IC$_{50}$ Determination	Human CIC-2 Channels in CHO Cells	*In vitro*		99933 (093-445)

Overview	Test Article: SEP-0002093, Eslicarbazepine, (R)-Licarbazepine, (b) (4) (b) (4) Oxcarbazepine, and Carbamazepine			
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study No. (Document No.)**
BIA 2-194, BIA 2-195, and Oxcarbazepine: Modulatory Effects on Recombinant GlyRα3 Currents Recorded from Stably Transfected CHO Cells	Human GlyRα3 Channels in CHO Cells	*In vitro*	(b) (4)	99963 (093-446)
BIA 2-194, BIA 2-195, and Oxcarbazepine: Effect on Glycine Transporters Type 1 and Type 2 (GlyT1 and GlyT2) Recorded from Transiently Transfected HEK293 Cells	Human Glycine Transporters in HEK293 Cells	*In vitro*		A0036 (093-447)
Effect of Eslicarbazepine Acetate, (S)-Licarbazepine, (R)-Licarbazepine, and Oxcarbazepine in Fully Kindled Mice	NMRI Mice	Intraperitoneal		SR-HP081119 (093-457)
Evaluation of BIA 2-093 and BIA 2-194 in the Maximal Electroconvulsive Shock Test in the Mouse	NMRI Mice	Oral		09.636/3 (093-458)

Overview	Test Article: SEP-0002093, Eslicarbazepine, (R)-Licarbazepine, (b) (4) (b) (4) Oxcarbazepine, and Carbamazepine			
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study No. (Document No.)**
Evaluation of Carbamazepine and Oxcarbazepine in the Maximal Electroconvulsive Shock Test in the Mouse	NMRI Mice	Oral	(b) (4)	09.645/2 (093-459)
Evaluation of BIA 2-093 and BIA 2-194 for Potential Anticonvulsant Activity Using the 6 Hz Psychomotor Test in the Mouse	NMRI Mice	Oral		09.511/2 (093-460)
Evaluation of Carbamazepine and Oxcarbazepine for Potential Anticonvulsant Activity Using the 6 Hz Psychomotor Test in the Mouse	NMRI Mice	Oral		09.529/1 (093-461)
Secondary Pharmacodynamics[b]				
Evaluation of BIA 2-093, BIA 2-194, BIA 2-059, and BIA 2-195 Using the Formalin (Late Phase) Test in the Mouse	NMRI Mice	Oral		05.130/2 (093-474)
Evaluation of BIA 2-093 Using the Formalin (Late Phase) Test in the Mouse	NMRI Mice	Oral		05.373/3 (093-475)
Safety Pharmacology[b]				
Evaluation of BIA 2-093 and BIA 2-194 Using the Primary Observation (Irwin) Test in the Mouse	NMRI Mice	Oral		09.637/2 (093-464)
Evaluation of BIA 2-093 and BIA 2-194 Using the Rotarod Test in the Mouse	NMRI Mice	Oral		09.638/2 (093-465)
Evaluation of Carbamazepine and Oxcarbazepine Using the Primary Observation (Irwin) Test in the Mouse	NMRI Mice	Oral		09.646/1 (093-466)
Evaluation of Carbamazepine and Oxcarbazepine Using the Rotarod Test in the Mouse	NMRI Mice	Oral		09.647/1 (093-467)
Evaluation of Carbamazepine and Oxcarbazepine Using the Modified Rotarod Test in the Mouse	NMRI Mice	Oral		09.761/1 (093-468)
Evaluation of BIA 2-093 and BIA 2-194 Using the Modified Rotarod Test in the Mouse	NMRI Mice	Oral		09.762/1 (093-469)

[a] CHO = Chinese Hamster Ovary; HEK = human embryonic kidney
[b] BIA 2-093 = eslicarbazepine acetate = SEP-0002093; BIA 2-194 = eslicarbazepine; BIA 2-195 = (R)-licarbazepine; (b) (4)

Toxicology

Overview[a]				Test Article: SEP-0002093 (or as otherwise noted)			
Type of Study	**Species and Strain[b]**	**Method of Administration**	**Duration of Dosing**	**Test Article and Doses or Concentrations[c]**	**GLP Compliance**	**Testing Facility**	**Study Number (Document)**
Chromosome aberration assay	Human peripheral blood lymphocytes	*In vitro*	NA	Eslicarbazepine (BIA 2-194): 10 to 5000 µg/mL	Yes	(b) (4)	789008 (093-884)
Bacterial reverse mutation (Ames)	*S. typhimurium* and *E. coli*	*In vitro*	NA	SEP-0002093: 17 to 5000 µg/plate	Yes		788973 (093-885)
Bacterial reverse mutation (Ames)	*S. typhimurium* and *E. coli*	*In vitro*	NA	SEP-0002093, Eslicarbazepine, (R)-Licarbazepine, and Oxcarbazepine: 17 to 5000 µg/plate	No		789013 (093-892)
Juvenile Toxicity	Dog, Beagle	Oral (Gavage)	28 days	SEP-0002093: 0, 25, 100, and 200 mg/kg/day	Yes		682001 (093-800)
Juvenile Toxicity	Dog, Beagle	Oral (Gavage)	10 months	SEP-0002093: 0, 40, 80, and 160 mg/kg/day	Yes		682002 (093-866)

3.2 Studies Not Reviewed: The following studies were not reviewed as they were abuse liability studies (to be reviewed by the Controlled Substances Staff), environmental toxicology studies, or primary pharmacodynamic studies that examined endpoints that were not relevant to the indication specified in the current NDA (sponsor's tables, below):

Type of Study	Test System	Method of Administration	Testing Facility	Study No. (Document No.)
Mouse Isolated Sciatic Nerve Study to Identify the Pain Transmitting Fibre Type Affected by BIA 2-194	Sciatic Nerve from BL6 CBF1 Mice	*In vitro*	(b) (4)	ZNA22818 (093-452)
Investigation of the Effects of Oxcarbazepine on Epileptiform Activity in Mouse Hippocampal Slices	Hippocampal Slices from CD1 Mice	*In vitro*		ZNA22889 (093-454)
A Study of the Site(s) of Action of BIA 2-194 on Ectopic Discharge in CCI Mice	BL6 CBF1 Mice	Intraperitoneal		ZNA22819 (093-453)
Secondary Pharmacodynamics[b]				
Evaluation of BIA 2-093, BIA 2-194 (b) (4) and BIA 2-195 Using the Formalin (Late Phase) Test in the Mouse	NMRI Mice	Oral		05.130/2 (093-474)
Evaluation of BIA 2-093 Using the Formalin (Late Phase) Test in the Mouse	NMRI Mice	Oral		05.373/3 (093-475)

Overview				Test Article: SEP-0002093 (or as otherwise noted)			
Type of Study	**Species and Strain**	**Method of Administration**	**Duration of Dosing**	**Test Article and Doses or Concentrations**	**GLP Compliance**	**Testing Facility**	**Study Number (Document)**
Range-Finding Study	Mouse, CD-1	Oral (Gavage)	21 days	Diazepam: 10, 30, 100, 300, and 500 mg/kg/day 10, 30, 100, 300, and 500 mg/kg b.i.d.	No	(b) (4)	(b) (4) AC-312348 (093-887)
Assessment of Dependence Potential	Mouse, CD-1	Oral (Gavage)	21 days	SEP-0002093: 250, 250/400, and 250/600 mg/kg/day[d] Diazepam: 100/300/500 and 100/300/500/750 mg/kg/day	Yes		(b) (4) AC-312349 (093-890)
Adsorption Testing	NA	*In vitro*	NA	SEP-0002093: 0.01 mg/mL	Yes		2733/0001 (093-875)
Respiration in Activated Sludge	NA	Static solution	3 hours	SEP-0002093: 0 to 1000 mg/L	Yes		33714 EAS (093-879)
Biodegradation in Sewage Sludge	NA	*In vitro*	28 days	SEP-0002093: 14.5 mg/L	Yes		33715 ECS (093-880)
Degradation in Water/ Sediment System	NA	*In vitro*	101 days	SEP-0002093: 113.8 µg/500 mL	No		00G0305/ 093077 (093-882)

Range-Finding Study	Mouse, CD-1	Oral (Gavage)	21 days	SEP-0002093: 100, 400, 600, 250/600, and 250/800 mg/kg/day[4]	No	(b) (4)	(b) (4) -312347 (093-886)

Overview				Test Article: SEP-0002093 (or as otherwise noted)			
Type of Study	**Species and Strain**	**Method of Administration**	**Duration of Dosing**	**Test Article and Doses or Concentrations**	**GLP Compliance**	**Testing Facility**	**Study Number (Document)**
Algal Growth Inhibition	Algae, *P.subcapitata*	Static solution	72 hours	SEP-0002093: 0 to 82.5 mg/L	Yes	(b) (4)	33713 EAA (093-878)
Acute Toxicity	Water flea, *D. magna*	Semi-static solution	48 hours	SEP-0002093: 0 to 102.3 mg/L	Yes		33712 EAD (093-877)
Reproduction Test	Water flea, *D. magna*	Semi-static solution	21 days	SEP-0002093: 0 to 88.99 mg/L	Yes		34364 ECD (093-881)
Acute Toxicity	Rainbow Trout, *O. mykiss*	Semi-static solution	96 hours	SEP-0002093: 0 to 98 mg/L	Yes		33711 EAP (093-876)
Early-Life Stage Toxicity	Rainbow Trout *O. mykiss*	Semi-static solution	91 days	SEP-0002093: 0 to 8.90 mg/L	Yes		34363 ECP (093-883)
Survival, Growth, and Emergence Tests	Non-biting midge *C. riparius*	Spiked water	28 days	Eslicarbazepine 0 to 100 mg/L	Yes		41004456 and 41102564 (093-893 and 093-894)

3.3 Previous Nonclinical Reviews Referenced

A) Toscano CD, Nonclinical Review, Filed in DARRTS 4/14/2010.
B) Freed, LM, Supervisory Pharmacologist Memo, Filed in DARRTS 4/28/2010.
C) Brown, PC, Tertiary Pharmacology Review, Filed in DARRTS 4/29/2010.

4 Pharmacology

4.1 Primary Pharmacology

The sponsor performed multiple *in vitro* studies to assess the activity of eslicarbazepine and related compounds (R-licarbazepine and oxcarbazepine) at ligand-gated ion channels such as voltage-gated sodium channels (VGSC), voltage-gated calcium channels, potassium channels, chloride channels, glutamate-activated ionotropic channels (AMPAR and NMDAR), and GABAergic receptors.

The results of the studies conducted with CHO cells transfected with rat or human sodium channels are provided in sponsor's table 2, below. As demonstrated in the sponsor's original NDA submission (received 3/30/2009) and sponsor's table 2 below, eslicarbazepine exhibits some activity at several human and rat VGSCs; however, eslicarbazepine was less potent than oxcarbazepine at the rat $Na_V1.2$ and human $Na_V1.3$ VGSCs.

Table 2: Effects of Eslicarbazepine, (R)-Licarbazepine, and Oxcarbazepine on Voltage-Gated Sodium Channels Expressed in CHO Cells

Channel[a]	IC_{50} for Inhibition of Channel Current (μM)[b]		
	Eslicarbazepine	(R)-Licarbazepine	Oxcarbazepine
Human $Na_V1.1$	937	1510	-
Rat $Na_V1.2$ (-80 mV)	829	780	456
Rat $Na_V1.2$ (-70 mV)	224	335	81
Human $Na_V1.3$ (-80 mV)	704	383	-
Human $Na_V1.3$ (-60 mV)	268	158	100
Human $Na_V1.7$	523	246	-

[a] Data are from the following documents: Human $Na_V1.1$ (093-435), Rat $Na_V1.2$ (-80 mV signifies a depolarization step from -80 mV to -10 mV) (093-436 and 093-473), Rat $Na_V1.2$ (-70 mV signifies a depolarization step from -70 mV to -10 mV) (093-470), Human $Na_V1.3$ (093-437) (-80 mV signifies a depolarization step from -80 mV to -10 mV), Human $Na_V1.3$ (093-472) (-60 mV signifies a depolarization step from -60 mV to -10 mV), Human $Na_V1.7$ (093-438)

[b] - = Not tested

HEK293 cells expressing $Ca_V3.2$ (T-type) or $Ca_V2.1$ channels (Studies 093-439 and 093-440, respectively) were incubated with BIA 2-194 (eslicarbazepine) or BIA 2-195 ((R)-licarbazepine). The IC_{50} values for BIA 2-194 and BIA 2-195 at the $Ca_V3.2$ (T-type) channel were 380 nM and 4.84 μM, respectively; the biphasic dose-response suggested more than one binding site (Study 093-439). There was minimal activity of either compound at the $Ca_V2.1$ channel when tested up to a concentration of 1 mM (Study 093-440). BIA 2-194 exhibited no activity at the $K_V7.2$ potassium channel when tested up to a concentration of 550 μM; the IC_{50} calculated for BIA 2-195 at this channel was 1.2 mM (Study 093-441). At concentrations of up to 1 mM, there was minimal activity of BIA 2-194 and BIA 2-195 at the endogenous potassium channels expressed in N1E-115 mouse neuroblastoma cells (Study 093-471) and no activity at AMPA channels (Study 093-442). BIA 2-194 inhibited NMDAR current by 22% at 500 μM and 42% at 1000 μM; BIA 2-195 inhibited NMDAR current by 18% and 28% at these concentrations,

respectively (Study 093-444). The positive control (10 µM AP-5) inhibited NMDA current by 75%. BIA 2-194 had a minimal effect on ClC-2 chloride channels when tested up to 500 µM; however, BIA 2-195 inhibited these channels with an IC_{50} of 464 µM (Study 093-445). The glycine receptor, GlyRα3, was inhibited by BIA 2-194 (IC_{50} ~1 mM), BIA 2-195 (IC_{50} ~1 mM), and oxcarbazepine (IC_{50} ~370 µM; Study 093-446); there was no effect of these compounds on the human glycine transporters, hGlyT1 and hGlyT2, when tested *in vitro* (Study 093-447).

In vivo studies were conducted to test the efficacy of BIA 2-093 (eslicarbazepine acetate), BIA 2-194, and other related compounds in fully kindled mice (Study 093-457), the maximal electroconvulsive shock test (MEST; Study 093-458), and the 6Hz psychomotor test (Study # 093-460, 093-461). In Study 093-457, male NMRI mice were implanted with kindling electrodes (near the amygdala) and given single i.p. doses of the test articles after a kindling paradigm (sponsor's table 2 below). BIA 2-093 (300 mg/kg) and oxcarbazepine (100 mg/kg) decreased the number of animals exhibiting seizure activity (sponsor's table 2, below). In the MEST assay (Study 093-458 & 093-459), male NMRI mice were given a single oral dose of BIA 2-093, BIA 2-194, oxcarbazepine, or carbamazepine one hour before delivery of electroconvulsive shock; BIA 2-093 and BIA 2-194 demonstrated a dose-dependent decrease in convulsions (sponsor's table 1, below).

Table 2:
Effects of eslicarbazepine acetate, (S)-licarbazepine, (*R*)-Licarbazepine & oxcarbazepine on seizure activity in fully kindled mice.

Dose (mg / kg)	Number of mice with seizure activity
Eslicarbazepine acetate (Bia 2-093)	
100	10 / 10
200	9 / 10
300	6 / 13 *
(*S*)-Licarbazepine (Bia 2-194)	
100	13 / 13
200	10 / 10
300	11 / 12
(*R*)-Licarbazepine (Bia 2-195)	
100	13 / 13
200	10 / 11
300	12 / 12
Oxcarbazepine	
10	13 / 13
30	12 / 13
100	5 / 11*

*P < 0.05 compared to Vehicle group.
The significance of differences in the number of animals showing seizure activity in response to electrical stimulation up to a maximum of 1200µA were analyzed using Fisher´s exact test.
Vehicle group: all animals showed seizure activity.

Table 1: Effects of BIA 2-093, BIA 2-194 and diazepam in the Maximal Electroconvulsive Shock (MES) Test in the mouse (12 mice per group)

TREATMENT (mg/kg) p.o. -60 min	NUMBER OF TONIC CONVULSIONS		PERCENT CHANGE FROM CONTROL
Vehicle	12		
BIA 2-093 (2.5)	11		-8%
BIA 2-093 (5)	12		0%
BIA 2-093 (10)	12		0%
BIA 2-093 (25)	10		-17%
BIA 2-093 (50)	6	+	-50%
BIA 2-093 (100)	3	+++	-75%
BIA 2-093 (150)	1	+++	-92%
BIA 2-194 (2.5)	12		0%
BIA 2-194 (5)	12		0%
BIA 2-194 (10)	11		-8%
BIA 2-194 (25)	7	+	-42%
BIA 2-194 (50)	2	+++	-83%
BIA 2-194 (100)	2	+++	-83%
BIA 2-194 (150)	0	+++	-100%
Diazepam (8)	1	+++	-92%

Fisher's Exact test: **no indication = not significant; + = $p < 0.05$; +++ = $p < 0.001$.**

Table 1: Effects of carbamazepine, oxcarbazepine and diazepam in the Maximal Electroconvulsive Shock (MES) Test in the mouse (12 mice per group)

TREATMENT (mg/kg) p.o. -60 min	NUMBER OF TONIC CONVULSIONS		PERCENT CHANGE FROM CONTROL
Vehicle	12		
Carbamazepine (1)	12		0%
Carbamazepine (2.5)	10		-17%
Carbamazepine (5)	11		-8%
Carbamazepine (10)	7	+	-42%
Carbamazepine (25)	3	+++	-75%
Carbamazepine (50)	0	+++	-100%
Carbamazepine (100)	0	+++	-100%
Oxcarbazepine (1)	12		0%
Oxcarbazepine (2.5)	12		0%
Oxcarbazepine (5)	10		-17%
Oxcarbazepine (10)	9		-25%
Oxcarbazepine (25)	1	+++	-92%
Oxcarbazepine (50)	0	+++	-100%
Oxcarbazepine (100)	1	+++	-92%
Diazepam (8)	0	+++	-100%

Fisher's Exact test: no indication = not significant; + = $p < 0.05$; +++ = $p < 0.001$.

In the 6 Hz psychomotor assay (Study #s 093-460 and 093-461), male NMRI mice were given a single oral dose of BIA 2-093, BIA 2-194, carbamazepine, oxcarbazepine, or the positive control (4 mg/kg diazepam) one hour before stimulation with a corneal electric shock. A dose-dependent decrease in seizure score and Straub tail score was observed in mice dosed with BIA 2-093, BIA 2-194, carbamazepine, and oxcarbazepine (sponsor's table 1, below).

Table 1: Effects of BIA 2-093, BIA 2-194 and diazepam in the 6 Hz Psychomotor Seizure model in the mouse (15 mice per group)
(Intensity: 24 mA)

TREATMENT (mg/kg) p.o. -60 min	SEIZURE SCORE				STRAUB TAIL SCORE	
	mean ± s.e.m.		p value	mean change		
Vehicle	0.8 ± 0.1				12	
BIA 2-093 (2.5)	0.3 ± 0.1	*	0.0251	-0.5	10	
BIA 2-093 (5)	0.2 ± 0.1	**	0.0036	-0.6	7	
BIA 2-093 (10)	0.2 ± 0.1	**	0.0029	-0.6	12	
BIA 2-093 (25)	0.2 ± 0.1	**	0.0036	-0.6	5	+
BIA 2-093 (50)	0.1 ± 0.1	**	0.0011	-0.7	7	
BIA 2-093 (100)	0.1 ± 0.1	***	0.0003	-0.7	3	++
BIA 2-093 (150)	0.1 ± 0.1	**	0.0011	-0.7	1	+++
BIA 2-194 (2.5)	0.7 ± 0.2	NS	0.5018	-0.1	13	
BIA 2-194 (5)	0.6 ± 0.2	NS	0.3000	-0.2	12	
BIA 2-194 (10)	0.4 ± 0.2	NS	0.0511	-0.4	13	
BIA 2-194 (25)	0.3 ± 0.1	*	0.0251	-0.5	8	
BIA 2-194 (50)	0.5 ± 0.2	NS	0.1758	-0.3	6	
BIA 2-194 (100)	0.1 ± 0.1	**	0.0011	-0.7	1	+++
BIA 2-194 (150)	0.4 ± 0.2	*	0.0381	-0.4	5	+
Diazepam (4)	0.1 ± 0.1	***	0.0003	-0.7	2	+++

Mann-Whitney U test (seizure score):
NS = Not Significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.
Fisher's Exact test (number of mice with Straub tail):
no indication = not significant; + = $p < 0.05$; ++ = $p < 0.01$; +++ = $p < 0.001$.

Table 1:Effects of carbamazepine, oxcarbazepine and diazepam in the 6 Hz Psychomotor Seizure model in the mouse (15 mice per group)
(Intensity: 24 mA)

TREATMENT (mg/kg) p.o. -60 min	SEIZURE SCORE				STRAUB TAIL SCORE	
	mean ± s.e.m.		p value	mean change		
Vehicle	0.6 ± 0.2				12	
Carbamazepine (1)	0.7 ± 0.2	NS	0.5122	+0.1	10	
Carbamazepine (2.5)	0.5 ± 0.1	NS	0.6041	-0.1	9	
Carbamazepine (5)	0.5 ± 0.2	NS	0.5122	-0.1	12	
Carbamazepine (10)	0.7 ± 0.2	NS	0.7446	+0.1	13	
Carbamazepine (25)	0.3 ± 0.1	NS	0.2327	-0.3	5	+
Carbamazepine (50)	0.0 ± 0.0	**	0.0012	-0.6	4	++
Carbamazepine (100)	0.1 ± 0.1	*	0.0204	-0.5	5	+
Oxcarbazepine (1)	0.8 ± 0.1	NS	0.3207	+0.2	13	
Oxcarbazepine (2.5)	0.7 ± 0.2	NS	0.9636	+0.1	14	
Oxcarbazepine (5)	0.7 ± 0.2	NS	0.5122	+0.1	10	
Oxcarbazepine (10)	0.7 ± 0.2	NS	0.7446	+0.1	10	
Oxcarbazepine (25)	0.2 ± 0.1	NS	0.0548	-0.4	6	
Oxcarbazepine (50)	0.1 ± 0.1	*	0.0204	-0.5	5	+
Oxcarbazepine (100)	0.1 ± 0.1	**	0.0059	-0.5	6	
Diazepam (4)	0.0 ± 0.0	**	0.0012	-0.6	1	+++

Mann-Whitney U test (seizure score):
NS = Not Significant; * = $p < 0.05$; ** = $p < 0.01$.
Fisher's Exact test (number of mice with Straub tail):
no indication = not significant; + = $p < 0.05$; ++ = $p < 0.01$; +++ = $p < 0.001$.

4.3 Safety Pharmacology

The sponsor performed several CNS safety pharmacology studies in which the effect of BIA 2-093 and BIA 2-194 was examined separately from oxcarbazepine and carbamazepine in identically designed studies.

A) Study Reports 093-464 & 093-466: "Primary Observation (Irwin) Test in the Mouse"

Male NMRI mice (n=3/group) were given a single dose of BIA 2-093 (25, 50, 100, 250, 400, 500 mg/kg), BIA 2-194 (25, 50, 100, 200, 250, 400 mg/kg), carbamazepine (CBZ; 10, 25, 50, 100, 200, 250, 400 mg/kg), or oxcarbazepine (OXC; 10, 25, 50, 100, 200, 250, 400 mg/kg) by oral gavage; the vehicle control was 0.2% hydroxypropylmethycellulose in distilled water. Assessment of the animals occurred at 15, 30, 60, 120, and 180 minutes, and 24 hours after dosing.

The dose range used for each of the test articles was adequate, as the findings ranged from mildly (e.g., increased reactivity to touch) to moderately adverse (e.g., loss of righting reflex, tremor, catalepsy, decreased respiration; sponsor's tables, below) with increasing dose; there were no deaths in either study. Although the dose response differed among test articles, similar clinical signs were observed in mice dosed with BIA 2-093, BIA 2-194, OXC, and CBZ. Convulsion was only observed in animals dosed with BIA 2-194 (one of three mice dosed with 400 mg/kg). There were no test article-related findings when the animals were assessed 24 hours after dosing.

BIA 2-093

Table 1: Effects of BIA 2-093 in the Primary Observation (Irwin) Test in the mouse (3 mice per group)

DOSES (mg/kg, p.o.)					
25	50	100	200	250	
↑ Reactivity to touch (3/3) 60' → 120'	↑ Reactivity to touch (3/3) 15' → 60'	↑ Reactivity to touch (3/3) 60' → 120'	Decreased activity + (3/3) at 60' Abnormal gait (rolling) (3/3) at 60' ↓ Fear / startle (1/3) at 60' ↑ Reactivity to touch (3/3) 15' → 60' Hypothermia + at 15' ++ at 30' +++ at 60'	Tremor (1/3) 60' → 180' Decreased activity + (3/3) at 30' + (1/3) at 60' ++ (2/3) at 60' + (1/3) at 120' ++ (1/3) 120' → 180' Abnormal gait (rolling) (1/3) at 30' (3/3) at 60' (1/3) 120' → 180' Motor incoordination (1/3) 120' → 180' Akinesia (1/3) 60' → 180' Catalepsy (1/3) at 60'	Loss of traction (2/3) at 60' (1/3) 120' → 180' ↓ Respiration (1/3) 60' → 120' ↓ Fear / startle (1/3) 60' → 120' ↑ Reactivity to touch (3/3) 60' → 120' (1/3) at 180' Ptosis (1/3) at 30' (2/3) at 60' (1/3) 120' → 180' Hypothermia + at 15' +++ 30' → 60' ++ at 120' + at 180'

DOSES (mg/kg, p.o.)					
400			500		
Tremor (1/3) at 60' (2/3) 120' → 180' Decreased activity + (3/3) 15' → 30' ++ (3/3) at 60' ++ (1/3) at 120' +++ (2/3) 120' → 180' + (1/3) at 180' Abnormal gait (rolling) (3/3) 15' → 60' (1/3) at 120' Motor incoordination (1/3) at 60' ↓ Abdominal muscle tone (3/3) 60' → 120' (2/3) at 180'	Akinesia (2/3) at 60' (3/3) at 120' (1/3) at 180' Catalepsy (2/3) at 60' (3/3) at 120' (1/3) at 180' Loss of traction (2/3) at 60' (3/3) at 120' (2/3) at 180' ↓ Respiration (3/3) 60' → 120' (2/3) at 180' ↓ Fear / startle (3/3) 60' → 120' (2/3) at 180'	↑ Reactivity to touch (3/3) 15' → 60' ↓ Reactivity to touch (3/3) at 120' (2/3) at 180' Ptosis (2/3) 60' → 180' Exophthalmia (1/3) at 60' Hypothermia + at 15' +++ 30' → 180'	Tremor (1/3) at 30' (2/3) at 60' (1/3) at 120' (2/3) at 180' Straub tail (1/3) at 120' Decreased activity + (3/3) at 15' ++ (3/3) 30' → 60' ++ (2/3) 120' → 180' +++ (1/3) at 120' + (1/3) at 180' Abnormal gait (rolling) (3/3) 15' → 60' (2/3) 120' → 180'	Motor incoordination (1/3) at 30' (2/3) at 60' (1/3) at 120' (2/3) at 180' ↓ Abdominal muscle tone (3/3) 30' → 120' (2/3) at 180' Loss of grasping (1/3) at 60' Akinesia (1/3) at 180' Catalepsy (2/3) 60' → 180' Loss of traction (3/3) 30' → 60' (2/3) 120' → 180'	↓ Respiration (3/3) 30' → 180' ↓ Fear / startle (3/3) 60' → 120' (2/3) at 180' ↓ Reactivity to touch (3/3) 60' → 120' (2/3) at 180' Ptosis (3/3) 30' → 120' (2/3) at 180' Hypothermia ++ at 15' +++ 30' → 180' Hyperthermia + at 24h Mydriasis + at 180'

(X/N) indicates the number of mice showing the symptoms.
+ = slight; ++ = moderate; +++ = marked.
': minutes.
h: hours.
↑: increase.
↓: decrease.

BIA 2-194

Table 2: Effects of BIA 2-194 in the Primary Observation (Irwin) Test in the mouse (3 mice per group)

DOSES (mg/kg, p.o.)					
25	50	100	200		
No change	↑ Reactivity to touch (3/3) 15' → 30'	Abnormal gait (rolling) (1/3) 30' → 60' ↑ Reactivity to touch (3/3) 15' → 180' Hypothermia + 15' → 120'	Tremor (1/3) at 30' (3/3) at 60' (2/3) at 120' (1/3) at 180' Decreased activity + (3/3) at 30' ++ (3/3) at 60' ++ (2/3) at 120' +++ (1/3) at 120' + (2/3) at 180' ++ (1/3) at 180' Abnormal gait (rolling) (3/3) 15' → 180' Motor incoordination (1/3) 60' → 120'	↓ Abdominal muscle tone (2/3) at 30' (3/3) at 60' (2/3) at 120' (1/3) at 180' Akinesia (3/3) 60' → 120' Loss of traction (1/3) at 30' (2/3) 60' → 120' (1/3) at 180' ↓ Fear / startle (2/3) at 60' (3/3) at 120' (1/3) at 180'	↑ Reactivity to touch (3/3) at 15' ↓ Reactivity to touch (3/3) at 60' (2/3) at 120' (1/3) at 180' Ptosis (2/3) 30' → 120' (1/3) at 180' Hypothermia ++ at 15' +++ 30' → 180'

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DOSES (mg/kg, p.o.)					
250		400		500	
Tremor (1/3) at 15' (1/3) 60' → 180' Decreased activity + (3/3) at 15' ++ (3/3) 30' → 60' ++ (1/3) at 120' +++ (2/3) at 120' ++ (2/3) at 180' +++ (1/3) at 180' Abnormal gait (rolling) (3/3) 15' → 180' Motor incoordination (2/3) at 120' (1/3) at 180' ↓ Abdominal muscle tone (1/3) at 15' (3/3) 30' → 180' Loss of grasping (1/3) at 60'	Akinesia (2/3) at 30' (3/3) 60' → 120' (2/3) at 180' Loss of traction (3/3) 30' → 180' ↓ Fear / startle (1/3) at 15' (3/3) 30' → 60' (2/3) 120' → 180' ↓ Reactivity to touch (3/3) 30' → 180' Ptosis (2/3) at 30' (1/3) at 60' (3/3) at 120' (2/3) at 180' Hypothermia +++ 15' → 180'	Convulsions (1/3) at 180' Tremor (2/3) at 30' (3/3) at 60' (1/3) at 120' (3/3) at 180' Decreased activity + (3/3) at 15' ++ (3/3) at 30' ++ (2/3) at 60' +++ (1/3) at 60' +++ (3/3) at 120' ++ (1/3) at 180' +++ (2/3) at 180' Abnormal gait (rolling) (1/3) at 15' (3/3) 30' → 180' Motor incoordination (2/3) at 60' (3/3) at 120' (2/3) at 180' ↓ Abdominal muscle tone (1/3) at 15' (3/3) 30' → 180'	Loss of grasping (1/3) 60' → 180' Akinesia (3/3) 30' → 60' (2/3) 120' → 180' Catalepsy (1/3) at 60' (2/3) at 120' (1/3) at 180' Loss of traction (3/3) 30' → 180' ↓ Fear / startle (3/3) 30' → 180' ↓ Reactivity to touch (3/3) 30' → 180' Ptosis (2/3) at 30' (3/3) 60' → 120' (2/3) at 180' Hypothermia +++ 15' → 180'	Tremor (1/3) 0 → 15' (2/3) at 15' (3/3) 30' → 60' (1/3) at 120' (2/3) at 180' Decreased activity + (1/3) at 15' ++ (2/3) at 15' +++ (3/3) 30' → 180' Abnormal gait (rolling) (3/3) → 60' (2/3) 120' → 180' (3/3) at 24h Motor incoordination (3/3) 30' → 60' (2/3) at 120' (1/3) at 180' ↓ Abdominal muscle tone (3/3) 15' → 180' Loss of grasping (2/3) 60' → 180'	Akinesia (3/3) 30' → 180' Catalepsy (3/3) 30' → 180' Loss of traction (2/3) at 15' (3/3) 30' → 180' ↓ Respiration (1/3) at 30' (3/3) 60' → 180' ↓ Fear / startle (3/3) 30' → 180' ↓ Reactivity to touch (3/3) 30' → 180' Ptosis (1/3) at 15' (3/3) 30' → 180' Loss of righting reflex (1/3) 120' → 180' Hypothermia +++ 15' → 180'

(X/N) indicates the number of mice showing the symptoms.
+ = slight; ++ = moderate; +++ = marked.
': minutes.
h: hours.
↑: increase.
↓: decrease.

CBZ

Table 1: Effects of carbamazepine in the Primary Observation (Irwin) Test in the mouse (3 mice per group)

DOSES (mg/kg, p.o.)					
10	25	50	100		
No change	**No change**	**Abnormal gait (rolling)** (1/3) at 30'	**Decreased activity** + (1/3) at 15' ++ (2/3) at 15' + (2/3) 30' → 60' ++ (1/3) 30' → 60' + (1/3) at 120'	**Loss of grasping** (1/3) 15' → 30'	**↓ Reactivity to touch** (1/3) 30' → 60'
		Hypothermia + at 30'	**Abnormal gait (rolling)** (3/3) 0 → 15' (2/3) at 15' (1/3) 30' → 60' (2/3) at 120'	**Akinesia** (1/3) at 30' **Loss of traction** (1/3) 15' → 60'	**Ptosis** (1/3) 15' → 30' **Hypothermia** +++ 15' → 30' ++ at 60' + at 120'
			Motor incoordination (1/3) 15' → 60'	**↓ Respiration** (3/3) at 15' (1/3) 30' → 60' **↓ Fear / startle** (1/3) 15' → 60'	

DOSES (mg/kg, p.o.)					
200		250		400	
Tremor (1/3) at 180'	**Loss of traction** (3/3) 15' → 180'	**Tremor** (1/3) at 120' (2/3) at 180'	**Catalepsy** (1/3) 60' → 120'	**Tremor** (1/3) 0 → 15'	**Akinesia** (2/3) 15' → 120' (1/3) at 180'
Decreased activity ++ (3/3) 15' → 60' ++ (1/3) 120' → 180' +++ (2/3) 120' → 180'	**↓ Respiration** (3/3) 15' → 120' (2/3) at 180'	**Decreased activity** ++ (3/3) at 15' ++ (2/3) 30' → 120' +++ (1/3) 30' → 120' ++ (1/3) at 180' +++ (2/3) at 180'	**Loss of traction** (3/3) 15' → 180' **↓ Respiration** (3/3) 15' → 180' **↓ Fear / startle** (3/3) 15' → 180'	**Straub tail** (1/3) 0 → 15' **Decreased activity** ++ (2/3) at 15' +++ (1/3) at 15' ++ (1/3) 30' → 120' +++ (2/3) 30' → 120' ++ (2/3) at 180' +++ (1/3) at 180'	**Catalepsy** (1/3) 30' → 60' **Loss of traction** (3/3) 15' → 60' (2/3) at 120' (1/3) at 180'
↓ Fear / startle (3/3) 15' → 180'	**↓ Reactivity to touch** (3/3) 15' → 180'	**Abnormal gait (rolling)** (3/3) 0 → 15'	**↓ Reactivity to touch** (3/3) 30' → 180'	**Abnormal gait (rolling)** (3/3) 0 → 15'	**↓ Respiration** (3/3) 15' → 120' (2/3) at 180'
Abnormal gait (rolling) (3/3) 0 → 15'	**Ptosis** (3/3) 15' → 180'	**Motor incoordination** (3/3) at 15' (2/3) 30' → 120' (1/3) at 180'	**Ptosis** (3/3) 30' → 180'	**Motor incoordination** (3/3) at 15' (1/3) 30' → 120' (2/3) at 180'	**↓ Fear / startle** (3/3) 15' → 180'
Motor incoordination (3/3) 15' → 60' (1/3) 120' → 180'	**Loss of righting reflex** (1/3) at 15'	**↓ Abdominal muscle tone** (3/3) 120' → 180'	**Loss of righting reflex** (1/3) 30' → 120'	**↓ Abdominal muscle tone** (3/3) 60' → 120' (2/3) at 180'	**↓ Reactivity to touch** (3/3) 15' → 120' (2/3) at 180'
↓ Abdominal muscle tone (3/3) 60' → 180'	**Hypothermia** +++ 15' → 180'	**Loss of grasping** (3/3) 15' → 60' (2/3) 120' → 180'	**Analgesia** (1/3) 30' → 60' **Hypothermia** +++ 15' → 180'	**Loss of grasping** (2/3) 15' → 120' (1/3) at 180'	**Ptosis** (2/3) 15' → 180' **Loss of righting reflex** (1/3) 15' → 120' **Hypothermia** +++ 15' → 180'
Loss of grasping (3/3) at 15' (1/3) 30' → 60' (2/3) 120' → 180'					
Akinesia (3/3) at 15' (1/3) 30' → 60' (2/3) 120' → 180'		**Akinesia** (3/3) 15' → 60' (2/3) 120' → 180'			

(X/N) indicates the number of mice showing the symptoms.
+ = slight; ++ = moderate; +++ = marked.
': minutes.
↓: decrease.

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OXC

Table 2: Effects of oxcarbazepine in the Primary Observation (Irwin) Test in the mouse (3 mice per group)

DOSES (mg/kg, p.o.)					
10	25	50		100	
No change	**No change**	**Abnormal gait (rolling)** (1/3) at 30' (2/3) at 60'	**Hypothermia** + at 30'	**Abnormal gait (rolling)** (3/3) 30' → 120' (1/3) at 180'	**Hypothermia** + at 15' ++ at 30' + at 60'

DOSES (mg/kg, p.o.)					
200		250		400	
Tremor (1/3) at 60' (2/3) at 120' (1/3) at 180' **Decreased activity** + (1/3) at 15' + (2/3) at 30' ++ (1/3) at 30' + (1/3) at 60' ++ (2/3) at 60' ++ (3/3) at 120' ++ (2/3) at 180' + (1/3) at 180' **Abnormal gait (rolling)** (1/3) at 15' (3/3) 30' → 180' **Motor incoordination** (1/3) at 180' **↓ Abdominal muscle tone** (1/3) at 30' (2/3) at 60' (3/3) 120' → 180'	**Loss of traction** (1/3) 30' → 60' (3/3) 120' → 180' **↓ Fear / startle** (1/3) at 60' (3/3) 120' → 180' **↓ Reactivity to touch** (1/3) at 60' (3/3) at 120' (2/3) at 180' **Ptosis** (2/3) 30' → 60' (1/3) at 120' (2/3) at 180' **Hypothermia** ++ at 15' +++ 30' → 180'	**Tremor** (1/3) at 60' (3/3) at 120' (2/3) at 180' **Decreased activity** + (2/3) at 15' → 30' ++ (1/3) at 30' ++ (3/3) 60' → 120' ++ (2/3) at 180' + (1/3) at 180' **Abnormal gait (rolling)** (2/3) at 15' (3/3) 30' → 180' **↓ Abdominal muscle tone** (2/3) at 30' (3/3) 60' → 180'	**Loss of traction** (2/3) at 30' (1/3) at 60' (3/3) 120' → 180' **↓ Fear / startle** (3/3) at 120' (2/3) at 180' **↓ Reactivity to touch** (3/3) at 120' (2/3) at 180' **Ptosis** (3/3) at 30' (1/3) at 60' (2/3) at 120' (1/3) at 180' **Hypothermia** ++ at 15' +++ 30' → 180'	**Tremor** (1/3) at 30' (2/3) at 60' → 180' **Decreased activity** + (3/3) at 15' ++ (2/3) 30' → 60' + (1/3) 30' → 60' +++ (1/3) at 120' ++ (2/3) at 120' +++ (3/3) at 180' **Abnormal gait (rolling)** (3/3) 15' → 180' **↓ Abdominal muscle tone** (2/3) at 30' (3/3) 60' → 180'	**Loss of traction** (1/3) at 30' (2/3) at 60' (3/3) at 120' (2/3) at 180' **↓ Fear / startle** (2/3) 60' → 180' **↓ Reactivity to touch** (2/3) 60' → 180' **Ptosis** (2/3) 30' → 60' (3/3) at 120' (2/3) at 180' **Hypothermia** +++ 15' → 180'

(X/N) indicates the number of mice showing the symptoms.
+ = slight; ++ = moderate; +++ = marked.
': minutes.
↓: decrease.

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B) Study Reports 093-465 & 093-467: "The Rotarod Test in the Mouse"

Male NMRI mice (n=10/group) were given a single dose of BIA 2-093 (0, 25, 50, 100, 200, 250, 400, 500 mg/kg), BIA 2-194 (0, 25, 50, 100, 200, 250, 400, 500 mg/kg), carbamazepine (CBZ; 0, 10, 25, 50, 100, 200, 250, 400 mg/kg), or oxcarbazepine (OXC; 0, 10, 25, 50, 100, 200, 250, 400 mg/kg) by oral gavage 60 minutes before testing on the Rotarod; the vehicle control was 0.2% hydroxypropylmethycellulose in distilled water. The positive control was 8 mg/kg diazepam administered orally.

The dose ranges used in this study were identical to those used in the Irwin test reviewed above. OXC exhibited the greatest potency in the Rotarod test with ~50% decrease in drop-off time, relative to controls, observed at 50 mg/kg (sponsor's tables, below). A similar effect was observed at 100 mg/kg BIA 2-194 and CBZ; a 67% decrease, relative to vehicle, was observed with BIA 2-093 (sponsor's tables, below). Overall, these studies demonstrated impairment of motor coordination in animals dosed with BIA 2-093, BIA 2-194, OXC, and CBZ. This finding is consistent with the gait disturbances observed in the Irwin test reviewed above.

BIA 2-093

Table 1: Effects of BIA 2-093 and diazepam in the Rotarod Test in the mouse (10 mice per group)

BIA 2-093 (mg/kg) p.o. -60 min	NUMBER OF MICE FALLING	DROP-OFF TIME (s)			
		mean ± s.e.m.		p value	% change
Vehicle	4	129.4 ± 21.8			
25	6	103.5 ± 22.8	NS	0.4230	-20%
50	7	90.9 ± 24.4	NS	0.2553	-30%
100	9	43.1 ± 16.7	**	0.0057	-67%
200	9	35.1 ± 16.7	**	0.0030	-73%
250	10 +	16.0 ± 3.4	***	<0.0001	-88%
400	10 +	8.5 ± 5.0	***	<0.0001	-93%
500	10 +	3.9 ± 0.9	***	<0.0001	-97%
DIAZEPAM 8 mg/kg p.o. -60 min	10 +	3.7 ± 1.0	***	<0.0001	-97%

Fisher's Exact test (number of mice falling): no indication = not significant; + = $p < 0.05$.
Student's t test: NS = Not Significant; ** = $p < 0.01$; *** = $p < 0.001$.

BIA 2-194

Table 2: Effects of BIA 2-194 and diazepam in the Rotarod Test in the mouse (10 mice per group)

BIA 2-194 (mg/kg) p.o. -60 min	NUMBER OF MICE FALLING	DROP-OFF TIME (s)			
		mean ± s.e.m.		p value	% change
Vehicle	4	133.1 ± 20.8			
25	7	100.6 ± 22.7	NS	0.3052	-24%
50	4	115.5 ± 26.4	NS	0.6069	-13%
100	8	59.9 ± 20.7	*	0.0225	-55%
200	9	25.5 ± 17.3	***	0.0009	-81%
250	10 +	7.4 ± 2.0	***	<0.0001	-94%
400	10 +	1.5 ± 0.2	***	<0.0001	-99%
500	10 +	2.1 ± 0.9	***	<0.0001	-98%
DIAZEPAM 8 mg/kg p.o. -60 min	10 +	3.1 ± 0.4	***	<0.0001	-98%

Fisher's Exact test (number of mice falling): no indication = not significant; + = $p < 0.05$.
Student's t test: NS = Not Significant; * = $p < 0.05$; *** = $p < 0.001$.

CBZ

Table 1: Effects of carbamazepine and diazepam in the Rotarod Test in the mouse (10 mice per group)

CARBAMAZEPINE (mg/kg) p.o. -60 min	NUMBER OF MICE FALLING	DROP-OFF TIME (s)			
		mean ± s.e.m.		p value	% change
Vehicle	1	172.0 ± 8.0			
10	4	138.6 ± 18.9	NS	0.1210	-19%
25	4	122.8 ± 23.5	NS	0.0625	-29%
50	5	120.8 ± 23.4	NS	0.0532	-30%
100	7 +	74.9 ± 28.4	**	0.0040	-56%
200	10 +++	21.4 ± 13.7	***	<0.0001	-88%
250	10 +++	1.9 ± 0.3	***	<0.0001	-99%
400	10 +++	3.0 ± 1.2	***	<0.0001	-98%
DIAZEPAM 8 mg/kg p.o. -60 min	10 +++	2.9 ± 0.8	***	<0.0001	-98%

Fisher's Exact test (number of mice falling): no indication = not significant; + = $p < 0.05$; +++ = $p < 0.001$.
Student's t test: NS = Not Significant; ** = $p < 0.01$; *** = $p < 0.001$.

OXC

Table 2: Effects of oxcarbazepine and diazepam in the Rotarod Test in the mouse (10 mice per group)

OXCARBAZEPINE (mg/kg) p.o. -60 min	NUMBER OF MICE FALLING	DROP-OFF TIME (s)			
		mean ± s.e.m.		p value	% change
Vehicle	4	131.5 ± 20.1			
10	7	87.8 ± 21.1	NS	0.1509	-33%
25	7	103.1 ± 21.3	NS	0.3448	-22%
50	8	58.9 ± 23.2	*	0.0297	-55%
100	9	34.8 ± 18.0	**	0.0021	-74%
200	10 +	16.9 ± 5.9	***	<0.0001	-87%
250	10 +	11.0 ± 3.3	***	<0.0001	-92%
400	10 +	6.6 ± 2.8	***	<0.0001	-95%
DIAZEPAM 8 mg/kg p.o. -60 min	10 +	12.3 ± 9.3	***	<0.0001	-91%

Fisher's Exact test (number of mice falling): no indication = not significant; + = $p < 0.05$.
Student's t test: NS = Not Significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

C) Study Reports 093-468 & 093-469: "The Modified Rotarod Test in the Mouse"

Mice were tested in a modified Rotarod study that included a habituation day during which untreated mice were placed on the Rotarod for 3 minutes. The following day, mice were dosed orally with the test article and were tested on the Rotarod 60 minutes after dosing. Male NMRI mice (n=10/group) were given a single dose of BIA 2-093 (0, 25, 50, 100, 200, 250, 400, 500 mg/kg), BIA 2-194 (0, 25, 50, 100, 200, 250, 400, 500 mg/kg), carbamazepine (CBZ; 0, 10, 25, 50, 100, 200, 250, 400 mg/kg), or oxcarbazepine (OXC; 0, 10, 25, 50, 100, 200, 250, 400 mg/kg) by oral gavage. The positive control was 8 mg/kg diazepam (p.o.) in these studies. The NOEL, as determined by a decrease in drop-off time relative to vehicle control, for the test articles was 25 mg/kg OXC, 50 mg/kg CBZ, 100 mg/kg BIA 2-093, and 200 mg/kg BIA 2-194 (sponsor's tables, below).

BIA 2-093

Table 1: Effects of BIA 2-093 and diazepam in the Modified Rotarod Test in the mouse (10 mice per group).

BIA 2-093 (mg/kg) p.o. -60 min on Day 2	NUMBER OF MICE FALLING	DROP-OFF TIME (s)			
		mean ± s.e.m.		p value	% change
Vehicle	1	168.2 ± 11.8			
25	3	160.1 ± 12.6	NS	0.6443	-5%
50	0	180.0 ± 0.0	NS	0.3306	+7%
100	1	165.9 ± 14.1	NS	0.9018	-1%
200	3	142.1 ± 22.8	NS	0.3101	-16%
250	5	119.3 ± 22.9	NS	0.0739	-29%
400	8 ++	53.1 ± 21.9	***	0.0002	-68%
500	10 +++	16.3 ± 8.0	***	<0.0001	-90%
DIAZEPAM 8 mg/kg p.o. -60 min on Day 2	10 +++	20.2 ± 11.9	***	<0.0001	-88%

Fisher's Exact test (number of mice falling): no indication = not significant; ++ = $p < 0.01$; +++ = $p < 0.001$.
Student's t test: NS = Not Significant; *** = $p < 0.001$.

BIA 2-194

Table 2: Effects of BIA 2-194 and diazepam in the Modified Rotarod Test in the mouse (10 mice per group).

BIA 2-194 (mg/kg) p.o. -60 min on Day 2	NUMBER OF MICE FALLING	DROP-OFF TIME (s)			
		mean ± s.e.m.		p value	% change
Vehicle	2	152.7 ± 18.8			
25	1	177.6 ± 2.4	NS	0.2053	+16%
50	1	167.1 ± 12.9	NS	0.5355	+9%
100	0	180.0 ± 0.0	NS	0.1636	+18%
200	2	160.2 ± 15.9	NS	0.7639	+5%
250	4	131.1 ± 21.2	NS	0.4553	-14%
400 #1	8 +	75.8 ± 25.1	*	0.0245	-50%
500 #2	10 +++	20.4 ± 10.5	***	<0.0001	-87%
DIAZEPAM 8 mg/kg p.o. -60 min on Day 2	7	76.6 ± 26.9	*	0.0323	-50%

Fisher's Exact test (number of mice falling): no indication = not significant; + = $p < 0.05$; +++ = $p < 0.001$.
Student's t test: NS = Not Significant; * = $p < 0.05$; *** = $p < 0.001$.

#1: clonic convulsions (3/10).
#2: clonic convulsions (4/10).

CBZ

Table 1: Effects of carbamazepine and diazepam in the Modified Rotarod Test in the mouse (10 mice per group).

CARBAMAZEPINE (mg/kg) p.o. -60 min on Day 2	NUMBER OF MICE FALLING	DROP-OFF TIME (s)			
		mean ± s.e.m.		p value	% change
Vehicle	0	180.0 ± 0.0			
10	0	180.0 ± 0.0	-	-	0%
25	4	137.5 ± 19.2	*	0.0396	-24%
50	1	162.2 ± 17.8	NS	0.3306	-10%
100	7 ++	66.3 ± 25.1	***	0.0003	-63%
200	7 ++	68.4 ± 27.1	***	0.0006	-62%
250	7 ++	72.9 ± 24.8	***	0.0004	-60%
400	10 +++	5.3 ± 1.5	***	<0.0001	-97%
DIAZEPAM 8 mg/kg p.o. -60 min on Day 2	9 +++	54.0 ± 17.7	***	<0.0001	-70%

Fisher's Exact test (number of mice falling):
no indication = not significant; ++ = $p < 0.01$; +++ = $p < 0.001$.

Student's t test:
NS = Not Significant; * = $p < 0.05$; *** = $p < 0.001$.

OXC

Table 2: Effects of oxcarbazepine and diazepam in the Modified Rotarod Test in the mouse (10 mice per group).

OXCARBAZEPINE (mg/kg) p.o. -60 min on Day 2	NUMBER OF MICE FALLING	DROP-OFF TIME (s)			
		mean ± s.e.m.		p value	% change
Vehicle	2	169.1 ± 7.4			
10	1	165.8 ± 14.2	NS	0.8390	-2%
25	1	170.1 ± 9.9	NS	0.9364	+1%
50	5	112.6 ± 23.1	*	0.0317	-33%
100	7	99.2 ± 20.7	**	0.0052	-41%
200	9 ++	44.9 ± 20.8	***	<0.0001	-73%
250	10 +++	11.8 ± 4.2	***	<0.0001	-93%
400	10 +++	5.3 ± 0.9	***	<0.0001	-97%
DIAZEPAM 8 mg/kg p.o. -60 min on Day 2	8 +	65.4 ± 22.0	***	0.0003	-61%

Fisher's Exact test (number of mice falling):
no indication = not significant; + = $p < 0.05$; ++ = $p < 0.01$; +++ = $p < 0.001$.

Student's t test:
NS = Not Significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Eslicarbazepine Acetate, eslicarbazepine, R-licarbazepine and oxcarbazepine: testing for mutagenic activity with Salmonella typhimurium TA100, TA 1537, TA98, and Escherichia coli WP2uvrA (screening assay)

Study no.:	093-892
Study report location:	EDR
Conducting laboratory:	(b) (4)
Date of study initiation:	5/31/09
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Purity information was not provided Eslicarbazepine acetate, Batch 25570-16 Eslicarbazepine, Batch 2070-2-1 R-Licarbazepine, Batch PC040414 Oxcarbazepine, Batch FO40003

Key Study Findings

- **There was no cytotoxicity or increase in the number of revertant colonies observed at any concentration of the test articles employed.**

Methods

Strains:	S. typhimurium TA 1535, TA100, TA1537, TA98; E. coli WPuvrA
Concentrations in definitive study:	17, 50, 167, 500, 1667, 5000 µg/plate
Positive control:	+ Human or Rat Liver S9: 2-Aminoanthracene (0.01-0.1 µg/plate) - Human Liver S9: Sodium azide (1 µg/plate; TA1535, TA100), 9-aminoacridine (80 µg/ plate; TA1537), 2-nitrofluorene (1 µg/plate, TA98), N-ethyl-N-nitrosoguanisine (2 µg/plate, *E. coli*)
Negative Control and Vehicle:	DMSO
Incubation & sampling time:	The direct plating method was employed in this study. Plates were incubated at 37^{o}C for 48-72 hours.

Results: There was no cytotoxicity (decrease in number of colonies) observed at any concentration of the test articles employed; thereby suggesting that the concentration range would be appropriate to use in the pivotal study. There was no test article-related increase in the number of revertant colonies.

Study title: Eslicarbazepine Acetate: testing for mutagenic activity with Salmonella typhimurium TA1535, TA100, TA1537, TA98, and Escherichia coli WP2uvrA

Study no.:	093-885
Study report location:	EDR
Conducting laboratory:	(b) (4)
Date of study initiation:	7/20/2010
GLP compliance:	Yes, USFDA GLP
QA statement:	Yes
Drug, lot #, and % purity:	Eslicarbazepine acetate, Batch 56816-3-12, Purity 100.2%

Key Study Findings

- **Eslicarbazepine acetate was negative in the Ames assay when incubated in the presence or absence of human hepatic S9 fraction.**

Methods

Strains:	S. typhimurium TA 1535, TA100, TA1537, TA98; E. coli WPuvrA
Concentrations in definitive study:	17, 50, 167, 500, 1667, 5000 µg/plate
Basis of concentration selection:	The highest concentration used in this study is based on the current regulatory guidance of 5 mg/plate. Cytotoxicity was not observed at any of the concentrations tested.
Positive control:	+ Human Liver S9: 2-Aminoanthracene (0.01-0.1 µg/plate) - Human Liver S9: Sodium azide (1 µg/plate; TA1535, TA100), 9-aminoacridine (80 µg/ plate; TA1537), 2-nitrofluorene (1 µg/plate, TA98), N-ethyl-N-nitrosoguanisine (2 µg/plate, *E. coli*)
Vehicle & Negative Control:	DMSO
Incubation & sampling time:	The assay was performed with both the direct plate incorporation method ("first mutation assay") and the pre-incubation method ("second mutation assay"). Plates were incubated at 37^{o}C for 48-72 hours.

Results: Eslicarbazepine acetate did not increase the number of revertant colonies in the presence or absence of human hepatic S9 fraction in the direct plate incorporation assay or the pre-incubation assay. The TA1535 culture in the pre-incubation assay was contaminated in the initial assay and was, therefore, repeated. Positive controls increased the number of revertant colonies, as expected. Overall, eslicarbazepine acetate was negative in the Ames assay.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Eslicarbazepine: Chromosomal aberration assay with human peripheral lymphocyte cultures *in vitro*

Study no.:	093-884
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	7/23/2010
GLP compliance:	Yes, USFDA
QA statement:	Yes
Drug, lot #, and % purity:	Eslicarbazepine (BIA 2-194), batch 2070-2-1, Purity= 100%

Key Study Findings

- **Eslicarbazepine, when incubated in the presence or absence of human hepatic S9 fraction, did not cause chromosomal aberrations.**

Methods

Cell line:	Human peripheral blood lymphocytes (hPBL)
Concentrations in definitive study:	156, 313, 625, 1250, 2500, 3300, 4200, 5000 µg/plate.
Vehicle & Negative control:	DMSO
Positive control:	+ human hepatic S9: Cyclophosphamide (10-50 µg/ml) - human hepatic S9: Mitomycin C (50-800 ng/ml)
Incubation & sampling time:	See sponsor table, below

Study Design:

S9 Mix	Cultures Established	Test	Treatment Period	Recovery Period (Includes 1 h wash)	Colcemid	Harvest
Presence of S9 mix	*ca* 48 h before exposure	Tests 1 and 2	0-5 h	5-26 h	26-29 h	29 h
Absence of S9 mix		Test 1	0-5 h	5-26 h	26-29 h	29 h
		Test 2	0-25 h	25-26 h	26-29 h	29 h
				25-50 h	50-53 h	53 h

Results:

Toxicity: Toxicity, in the form of cell lysis, was observed at the following concentrations of eslicarbazepine; the concentration ranges at which chromosomal aberration was assessed are provided for each condition:

Test 1 +S9 (5 hour treatment) ≥ 2500 µg/ml; range = 625, 1250, 2500 µg/ml
Test 1 –S9 (5 hour treatment) = 5000 µg/ml; range = 625, 1250, 2500 µg/ml
Test 2 +S9 (5 hour treatment) ≥ 3300 µg/ml; range = 2500, 3300, 4200, 5000 µg/ml
Test 2 –S9 (5 hour treatment) ≥ 3300 µg/ml; range = 625, 1250, 2500, 3300 µg/ml
Test 2 –S9 (24 hour treatment) ≥ 3300 µg/ml; range = 625, 1250, 2500, 3300 µg/ml

The mitotic index, relative to control, was > 50% at the concentrations in which cell lysis

was observed, except for in Test 2 in the absence of S9. Overall, the concentrations chosen to assess for chromosomal aberration were adequate.

Chromosomal Aberration: There was no concentration-dependent increase in chromosomal aberrations observed in the presence or absence of human hepatic S9 fraction.

9 Reproductive and Developmental Toxicology

9.4 Juvenile Toxicology

Study title: A 28-day oral (gavage) range-finding toxicity study of BIA 2-093 in juvenile beagle dogs.

Study no.:	093-800
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	4/15/2008
GLP compliance:	Yes, US FDA GLP
QA statement:	Yes
Drug, lot #, and % purity:	Eslicarbazepine acetate (BIA 2-093), Lot 25570-1-3, Purity 100%

Key Study Findings:

- **The NOAEL in this study was 25 mg/kg. At the HD (200 mg/kg), one animal was euthanized *in extremis*; the MTD in this study was 100 mg/kg.**
- **The main test article-related findings in this study were clinical signs and CNS signs in the neurobehavioral assessment.**

Methods

Doses:	0, 25, 100, 200 mg/kg
Frequency of dosing:	Once daily from PND 21 until PND 49
Dose volume:	5 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% w/v aqueous hydroxypropylmethycellulose
Species/Strain:	Beagle dog; (b) (4)
Age:	F1 animals were PND 11-14 at receipt; dosing commenced at PND 21 and ended on PND 48.
Number/Sex/Group:	See sponsor's table below
Study design:	See sponsor's figure below
Deviation from study protocol:	Deviations were minor and did not affect the validity of the study.

Study Design:

Toxicology Groups (b) (4) -682001M)

Group Number	Test Article	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Number of F_1 Animals[a] Males	Females
1	Vehicle Control	0	5	4	4
2	BIA 2-093	25	5	4	4
3	BIA 2-093	100	5	4	4
4	BIA 2-093	200	5	4	5

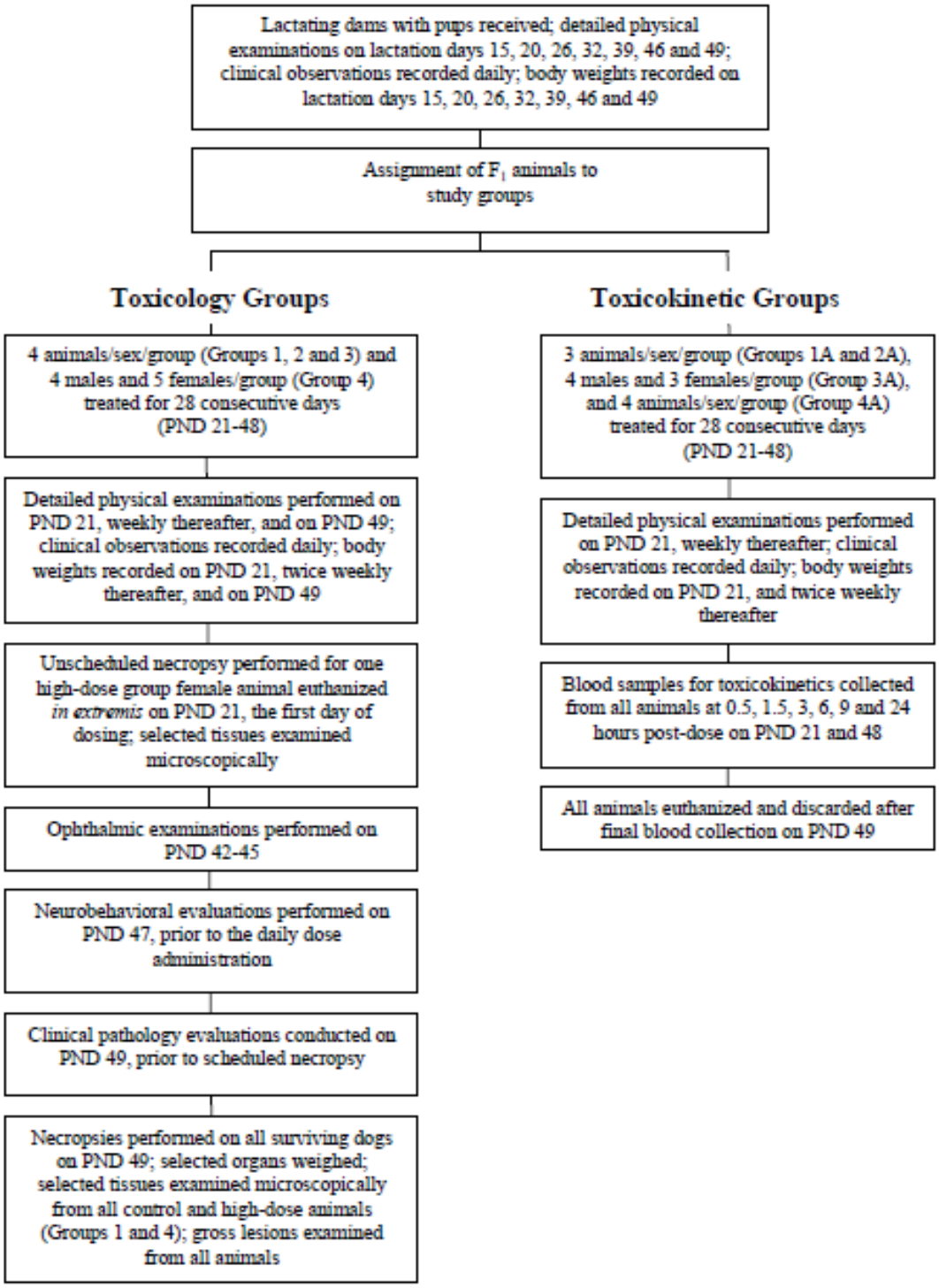
Toxicokinetic Groups (b) (4) -682001A)

Group Number	Test Article	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Number of F_1 Animals[b] Males	Females
1A	Vehicle Control	0	5	3	3
2A	BIA 2-093	25	5	3	3
3A	BIA 2-093	100	5	4	3
4A	BIA 2-093	200	5	4	4

[a] = All surviving F_1 pups in the Toxicology Groups were euthanized at the scheduled necropsy on PND 49.

[b] = All Toxicokinetic F_1 pups/sex/group were euthanized by sodium pentobarbital following the collection of the final toxicokinetic time point on PND 49.

3. STUDY DESIGN



Results:

Dosing Formulation: Dosing formulations were within 85% to 115% of the nominal concentration.

Mortality & Clinical Signs: One HDF (#5166-03) was euthanized *in extremis* on PND 21 after exhibiting hypoactivity, intermittent tremors, labored respiration, and prostration, which were noted to occur as early as 30 minutes after dosing. This death was considered to be test article-related.

There were no test article-related clinical signs observed in animals dosed with 25 mg/kg (sponsor's tables, below). Intermittent tremors, impaired muscle coordination, hypoactivity, and vocalization were observed in a limited number of animals dosed with 100 mg/kg. Intermittent tremors, continuous tremors, intermittent convulsions, continuous convulsions, impaired muscle coordination, impaired equilibrium, hypoactivity, vocalization, clear material around muzzle, pale gums, rigid muscle tone, and labored respiration occurred at a higher incidence in animals dosed with 200 mg/kg, relative to other dose groups.

	Toxicology Animals								Toxicokinetic Animals							
	Males				Females				Males				Females			
Dose Level (mg/kg/day)	0	25	100	200	0	25	100	200	0	25	100	200	0	25	100	2 0 0
No. of Dogs	4	4	4	4	4	4	4	5	3	3	3	3	4	3	4	4
Prostrate																
Detailed Physicals	-	-	-	-	-	-	-	1/1	-	-	-	-	-	-	-	-
Tremors, Intermittent																
Detailed Physicals	-	-	-	-	-	-	-	1/1	-	-	-	-	-	-	-	-
0.5 hour post-dose	-	-	-	1/1	-	-	-	1/1	-	-	-	2/2	-	-	-	3/2
1 hour post-dose	-	-	2/1	2/2	-	-	1/1	3/2	-	-	-	4/1	-	-	-	4/3
2 hours post-dose	-	-	-	6/2	-	-	1/1	6/3	-	-	-	1/1	-	-	-	5/2
3-4 hours post-dose	-	-	-	3/2	-	-	-	6/3	-	-	-	4/2	-	-	-	4/3
Unsch. Obs (>37-<45 minutes)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Unsch. Obs (>75-<105 minutes)	-	-	-	-	-	-	-	-	-	-	1/1	1/1	-	-	-	-
Unsch. Obs (>135-<180 minutes)	-	-	1/1	-	-	-	-	-	-	-	-	-	-	-	-	2/2
Unsch. Obs (>240 minutes)	-	-	-	2/2	-	-	-	1/1	-	-	-	1/1	-	-	-	1/1
Tremors, Continuous																
0.5 hour post-dose	-	-	-	-	-	-	-	1/1	-	-	-	2/2	-	-	-	-
1 hour post-dose	-	-	-	1/1	-	-	-	1/1	-	-	-	9/2	-	-	-	1/1
2 hours post-dose	-	-	-	1/1	-	-	-	1/1	-	-	-	1/1	-	-	-	2/1
3-4 hours post-dose	-	-	-	2/2	-	-	-	1/1	-	-	-	2/2	-	-	-	1/1
Unsch. Obs (>37-<45 minutes)	-	-	-	-	-	-	-	-	-	-	-	1/1	-	-	-	-
Unsch. Obs (>75-<105 minutes)	-	-	-	-	-	-	-	1/1	-	-	-	-	-	-	-	-
Convulsions, Intermittent																
Unsch. Obs (>75-<105 minutes)	-	-	-	-	-	-	-	-	-	-	-	1/1	-	-	-	-
Convulsions, Continuous																
2 hours post-dose	-	-	-	-	-	-	-	1/1	-	-	-	-	-	-	-	-

- = Not noted; Unsch. Obs = Unscheduled observations

	Main Study Animals								Toxicokinetic Animals							
	Males				Females				Males				Females			
Dose Level (mg/kg/day)	**0**	**25**	**100**	**200**	**0**	**25**	**100**	**200**	**0**	**25**	**100**	**200**	**0**	**25**	**100**	**200**
No. of Dogs	4	4	4	4	4	4	4	5	3	3	3	3	4	3	4	4
Impaired Muscle Coordination																
0.5 hour post-dose	-	-	-	-	-	-	-	2/2	-	-	1/1	-	-	-	-	-
1 hour post-dose	-	-	1/1	1/1	-	-	1/1	8/3	-	-	-	3/3	-	-	-	4/3
2 hours post-dose	-	-	-	8/3	-	-	1/1	9/3	-	-	-	6/3	-	-	-	7/3
3-4 hours post-dose	-	-	-	1/1	-	-	-	4/2	-	-	-	1/1	-	-	-	2/1
Unsch. Obs (>75-<105 minutes)	-	-	-	1/1	-	-	1/1	1/1	-	-	-	-	-	-	-	-
Impaired Equilibrium																
3-4 hours post-dose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2/2
Hypoactivity																
0.5 hour post-dose	-	-	-	4/2	-	-	-	7/4	-	-	-	3/2	-	-	-	3/2
1 hour post-dose	-	-	-	6/3	-	-	1/1	10/4	-	-	-	18/3	-	-	-	9/4
2 hours post-dose	-	-	-	10/2	-	-	1/1	12/3	-	-	-	1/1	-	-	1/1	10/2
3-4 hours post-dose	-	-	-	12/3	-	-	-	23/5	-	-	-	9/3	-	-	1/1	17/4
Unsch. Obs (>37-<45 minutes)	-	-	-	-	-	-	-	-	-	-	-	1/1	-	-	-	-
Unsch. Obs (>75-<105 minutes)	-	-	-	4/2	-	-	-	3/2	-	-	1/1	3/2	-	-	-	1/1
Unsch. Obs (>135-<180 minutes)	-	-	-	0/0	-	-	-	-	-	-	-	1/1	-	-	-	2/2
Unsch. Obs (>240 minutes)	-	-	-	1/1	-	-	-	-	-	-	-	-	-	-	-	1/1

- = Not noted; Unsch. Obs = Unscheduled observations

	Main Study Animals								Toxicokinetic Animals							
	Males				Females				Males				Females			
Dose Level (mg/kg/day)	**0**	**25**	**100**	**200**	**0**	**25**	**100**	**200**	**0**	**25**	**100**	**200**	**0**	**25**	**100**	**200**
No. of Dogs	4	4	4	4	4	4	4	5	3	3	3	3	4	3	4	4
Vocalization																
0.5 hour post-dose	-	-	-	1/1	-	-	-	4/4	-	-	-	3/2	-	-	-	2/2
1 hour post-dose	-	-	1/1	1/1	-	-	-	5/3	-	-	-	8/2	-	-	-	3/1
2 hours post-dose	-	-	-	6/2	-	-	1/1	2/1	-	-	-	-	-	-	-	10/3
3-4 hours post-dose	-	-	-	5/2	-	-	-	3/2	-	-	-	4/1	-	-	-	4/1
Unsch. Obs (>37-<45 minutes)	-	-	-	-	-	-	-	-	-	-	-	1/1	-	-	-	-
Unsch. Obs (>75-<105 minutes)	-	-	-	2/1	-	-	1/1	2/2	-	-	-	1/1	-	-	-	1/1
Unsch. Obs (>135-<185 minutes)	-	-	-	1/1	-	-	-	-	-	-	-	1/1	-	-	-	-
Unsch. Obs (>240 minutes)	-	-	-	1/1	-	-	-	1/1	-	-	-	1/1	-	-	-	-
Clear material around mouth																
At the time of dosing	-	-	-	24/2	-	-	1/1	16/4	-	-	-	15/3	-	-	-	4/3
0.5 hour post-dose	-	-	-	2/1	-	-	-	4/3	-	-	-	2/1	-	-	-	2/2
1 hour post-dose	-	-	-	2/2	-	-	-	3/2	-	-	-	3/2	-	-	-	1/1
2 hours post-dose	-	-	-	4/2	-	-	-	6/2	-	-	-	2/2	-	-	1/1	3/2
3-4 hours post-dose	-	-	-	4/3	-	-	-	6/3	-	-	-	2/2	-	-	1/1	1/1
Unsch. Obs (>75-<105 minutes)	-	-	-	2/1	-	-	-	1/1	-	-	-	1/1	-	-	-	1/1
Unsch. Obs (>135-<180 minutes)	-	-	-	-	-	-	-	-	-	-	-	1/1	-	-	-	1/1

- = Not noted; Unsch. Obs = Unscheduled observations

	Main Study Animals								Toxicokinetic Animals							
	Males				Females				Males				Females			
Dose Level (mg/kg/day)	**0**	**25**	**100**	**200**	**0**	**25**	**100**	**200**	**0**	**25**	**100**	**200**	**0**	**25**	**100**	**200**
No. of Dogs	4	4	4	4	4	4	4	5	3	3	3	3	4	3	4	4
Pale gums																
Detailed Physicals	-	-	-	-	-	-	-	1/1	-	-	-	-	-	-	-	-
0.5 hour post-dose	-	-	-	2/2	-	-	-	2/2	-	-	-	3/1	-	-	-	-
1 hour post-dose	-	-	-	2/2	-	-	-	3/2	-	-	-	6/2	-	-	-	4/4
2 hours post-dose	-	-	-	6/2	-	-	-	8/2	-	-	-	-	-	-	-	8/3
3-4 hours post-dose	-	-	-	9/3	-	-	-	15/5	-	-	-	8/3	-	-	-	9/4
Unsch. Obs (>75-<105 minutes)	-	-	-	3/2	-	-	-	2/2	-	-	-	2/2	-	-	-	1/1
Unsch. Obs (>135-<180 minutes)	-	-	-	-	-	-	-	-	-	-	-	1/1	-	-	-	2/2
Rigid Muscle Tone																
2 hours post-dose	-	-	-	-	-	-	-	1/1	-	-	-	-	-	-	-	-
Labored respiration																
0.5 hour post-dose	-	-	-	-	-	-	-	1/1	-	-	-	-	-	-	-	-
1 hour post-dose	-	-	-	-	-	-	-	-	-	-	-	1/1	-	-	-	-
3-4 hours post-dose	-	-	-	-	-	-	-	1/1	-	-	-	-	-	-	-	-
Unsch. Obs (>75-<105 minutes)	-	-	-	-	-	-	-	1/1	-	-	-	-	-	-	-	-

- = Not noted; Unsch. Obs = Unscheduled observations

Body Weights: There were no test article-related effects on absolute BW or BW gain.

Neurobehavioral Assessment: When performed on PND 47 before the daily dose was administered, neurobehavioral assessment included observation of home cage activity (general posture, head posture, tremors/convulsions, salivation, lacrimation palpebral closure), and open field/ functional observations (gait, behavior, respiration rate, perineal reflex, rectal temperature, cliff avoidance, pinna reflex, pupillary reflex, pupillary size, nystagmus, palpebral reflex, righting reflex, triceps reflex, patellar reflex, pinch reflex, proprioceptive positioning, posterior extension thrust, wheel barrowing, hemistand, capillary refill time, gag reflex, auditory response, menace reflex).

One HDF (5173-04) exhibited ataxia and absence of proprioceptive positioning, wheel barrowing, and hemistand walking. One LDM and one HDM did not exhibit the menace reflex.

Clinical Pathology, Hematology and Urinalysis: When conducted on samples collected on PND 49 just prior to necropsy, mean platelet counts were increased by 46% in HDF, relative to control. Reticulocyte count was decreased in all female dose groups in a dose-dependent manner (-17%, -19%, -35%). There were no other hematology findings that occurred in a dose-dependent or test article-related manner.

A slight dose-related increase in ALP was observed in all male dose groups (18%, 20%, 39%) and in HDF (38%). ALT was increased in all female dose groups in a dose-related manner (17%, 27%, 80%); GGT was increased by 2-fold in HDF. Cholesterol levels were increased in a dose-dependent manner in all female dose groups (9.2%, 31%, 70%). There were no test article-related findings on urinalysis parameters.

Ophthalmic Examination: When performed on PND 42-45, bilateral retinal dysplasia was observed in 1/5 HDM. A histopathology correlate for this finding was not observed in this animal.

Organ Weights: Absolute and BW-corrected liver weights were slightly increased in all male dose groups in a dose-dependent manner (sponsor's table, below). Adrenal gland weight (absolute and BW-relative) was increased in HDMs.

TABLE 16 (PND 49 NECROPSY)
A 28-DAY ORAL STUDY OF BIA 2-093 IN JUVENILE DOGS
SUMMARY OF ORGAN WEIGHTS AND RELATIVE ORGAN WEIGHTS

PROJECT NO. (b)(4)-682001M
SPONSOR:BIAL-PORTELA & CA
PAGE

GROUP:	0 MG/KG/DAY	MALES 25 MG/KG/DAY	100 MG/KG/DAY	200 MG/KG/DAY
ADRENAL GLANDS (G)				
MEAN	0.3118	0.3507	0.3271	0.4123
% DIFFERENCE		12.5	4.9	32.2
S.D.	0.10296	0.05734	0.05911	0.07914
S.E.	0.05148	0.02867	0.02955	0.03957
N	4	4	4	4
ADRENAL GLANDS (G/100 G FINAL BODY WEIGHT)				
MEAN	0.011	0.013	0.012	0.016
% DIFFERENCE		18.2	9.1	45.5
S.D.	0.0024	0.0017	0.0008	0.0034
S.E.	0.0012	0.0009	0.0004	0.0017
N	4	4	4	4
ADRENAL GLANDS (G/100 G BRAIN)				
MEAN	0.589	0.680	0.599	0.769
% DIFFERENCE		15.4	1.7	30.6
S.D.	0.1954	0.1295	0.0399	0.1317
S.E.	0.0977	0.0647	0.0200	0.0658
N	4	4	4	4
LIVER (G)				
MEAN	121.23	131.82	135.04	140.73
% DIFFERENCE		8.7	11.4	16.1
S.D.	21.326	13.240	20.459	27.928
S.E.	10.663	6.620	10.230	13.964
N	4	4	4	4
LIVER (G/100 G FINAL BODY WEIGHT)				
MEAN	4.354	4.681	5.056*	5.425**
% DIFFERENCE		7.5	16.1	24.6
S.D.	0.2803	0.2550	0.2299	0.4425
S.E.	0.1401	0.1275	0.1149	0.2212
N	4	4	4	4
LIVER (G/100 G BRAIN)				
MEAN	[illegible]	[illegible]	[illegible]	[illegible]
% DIFFERENCE		11.2	8.1	14.7
S.D.	42.9618	29.2249	15.7852	49.8212
S.E.	21.4809	14.6125	7.8926	24.9106
N	4	4	4	4

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Gross Pathology: There were no test article-related findings in the gross pathology assessment.

Histopathology: Adequate battery: No, nasal cavity/turbinates not examined; Signed and Dated Report: Yes; Peer Review: Yes. There were no test article-related findings.

Toxicokinetics: The main metabolite in dogs was BIA 2-194 (eslicarbazepine) and accounted for ~75% of the systemic exposure to BIA 2-093-related compounds; 12% of the exposure was accounted for by oxcarbazepine and the remaining exposure was due to the parent compound.

Text Table 4: MEAN TOXICOKINETIC RESULTS FOR BIA 2-093, BIA 2-194, AND OXCARBAZEPINE IN MALE JUVENILE DOGS

BIA 2-093 (mg/kg/day)	$AUC_{0.5-t}$ (ng•h/mL)		Metabolite/ Parent Ratio*		$AUC_{0.5-24h}$ (ng•h/mL)		C_{max} (ng/mL)		t_{max} (h)	
	PND 21	PND 48	PND 21	PND 48	PND 21	PND 48	PND 21	PND 48	PND 21	PND 48
Males	BIA 2-093 Results									
25	2890	1070	NA	NA	3030	1170	2150	1310	0.83	0.50
100	10200	4380	NA	NA	13000	4620	5710	4970	1.0	0.50
200	29600	7930	NA	NA	36300	9310	14100	6250	1.6	0.50
	BIA 2-194 Results									
25	15800	6480	5.4	6.0	16800	6810	5330	4500	1.2	0.50
100	105000	55700	10	13	117000	56800	24800	27100	1.9	1.0
200	356000	91200	13	12	356000	96600	48500	32300	1.9	1.0
	Oxcarbazepine Results									
25	3890	941	1.3	0.88	4520	1110	948	537	2.5	0.50
100	17300	6750	1.7	1.5	22000	7210	3390	3020	3.0	1.5
200	66900	12200	2.3	1.7	66900	13100	6290	3900	5.3	1.9

N=3 at 25 and N=4 at 100 mg/kg/day and 200 mg/kg/day.
*Ratio of Metabolite $AUC_{0.5-t}$ /BIA 2-093 $AUC_{0.5-t}$
NA = Not applicable

Study title: A 10-month oral (gavage) toxicity study of BIA 2-093 in juvenile beagle dogs with a 2-month recovery period

Study no.:	093-866
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	8/6/2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BIA 2-093, Lot #25570-1-4, 100% BIA 2-093, Lot #25570-1-6, 100%

Key Study Findings

- **The NOAEL in this study was < 40 mg/kg (at 40 mg/kg, BIA 2-093= 4,870 ng*hr/ml; BIA 2-194= 33,800 ng*hr/ml; OXC= 8,140 ng*hr/ml).**
- **Deaths occurred at all dose levels. Bone marrow depletion (1/2 LD, 2/2 MD, 3/4 HD; one HD not assessed) was observed in most and lymphoid tissue depletion was observed in all of the early decedents.**
- **Clinical signs were common at > 40 mg/kg and consisted of impaired coordination and equilibrium, tremors, and hypoactivity. Convulsions, labored respiration, rigidity, and nystagmus were observed at the HD.**
- **Neurobehavioral assessment in an FOB and an expanded CNS and PNS histopathology assessment found no test article-related effects.**
- **ALP, GGT, cholesterol, and triglycerides were elevated in all dose groups.**
- **Bone mineral content, bone area, and bone mineral density were decreased in females only.**
- **BIA 2-194, the main human metabolite of BIA 2-093, accounted for 72-85% of the systemic exposure to BIA 2-093-related material in males and females.**

Methods

Doses:	0, 40, 80, 160 mg/kg
Frequency of dosing:	Once daily from PNDs 21 through 328
Dose volume:	5 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% w/v aqueous hydroxypropylmethylcellulose (HPMC)
Species/Strain:	Beagle dogs; (b) (4)
Number/Sex/Group:	See sponsor's table, below.
Study design:	See sponsor's flowchart, below.
Deviation from study protocol:	Deviations did not compromise the integrity of the study.

Study Design: The sponsor provided the table below as justification for the starting date of dosing in pups (PND 21). Furthermore, the Division agreed to the design of the juvenile study in the meeting minutes for a teleconference between the sponsor and the division that occurred on 11/13/2008 (minutes filed in DARRTS under IND 67466 on 1/12/2009).

Age Range	Human	Dogs
Pre-term newborn infants	<38 weeks gestation	<0.5 weeks old
Neonates	0 - 27 days old	0.5 - 3 weeks old
Infant / Toddler	28 days - 23 months old	3 - 6 weeks old
Child	2 - 11 years old	6 - 20 weeks old
Adolescent	12 - 16 years old	20 - 28 weeks old

Source: Modified from Guidance for Industry: E11 Clinical Investigation of Medicinal Products in the Pediatric Population (2000); and from Buelke-Sam, J., Postnatal Evaluation in Development and Juvenile Toxicity Studies, presented at the Henry Stewart Conference; Meeting FDA, EPA, OECD and ICH Regulatory Requirements for Reproductive Toxicology Data Submissions. Georgetown University Conference Center, Washington, DC, 2002.

Toxicology Groups (b) (4)-682002M)

Group Number	Treatment	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Number of F_1 Animals [a] Males	Females
1	Vehicle Control	0	5	8	8
2	BIA 2-093	40	5	8	8
3	BIA 2-093	80	5	8	8
4	BIA 2-093	160	5	8	9

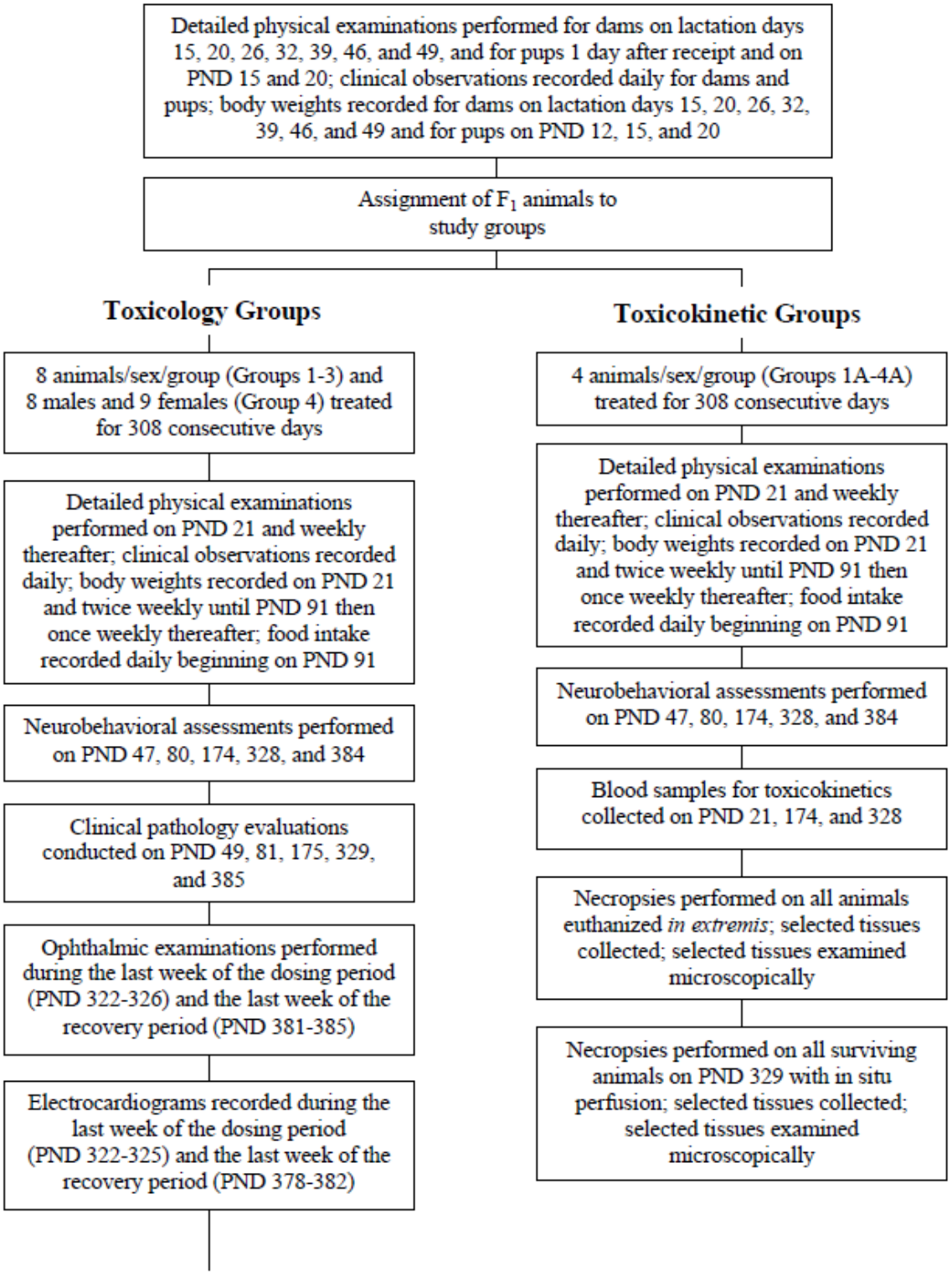
Toxicokinetic Groups (b) (4)-682002A)

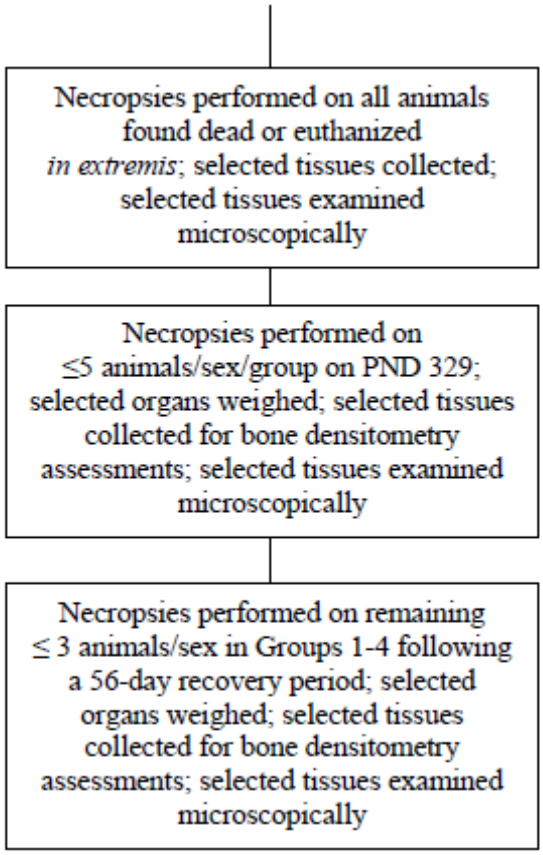
Group Number	Treatment	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Number of F_1 Animals [b] Males	Females
1A	Vehicle Control	0	5	4	4
2A	BIA 2-093	40	5	4	4
3A	BIA 2-093	80	5	4	4
4A	BIA 2-093	160	5	4	4

[a] = Up to 5 F_1 pups/sex/group in the toxicology groups were eligible for assignment to the scheduled primary necropsy on PND 329. Up to 3 animals/sex/group were eligible for assignment to a 56-day recovery and subsequent necropsy on PND 385.

[b] = All toxicokinetic F_1 pups/sex/group were subjected to whole-body perfusion at the primary necropsy on PND 329 following the last blood collection.

3. STUDY DESIGN





Dosing Formulations: Dosing formulations were 97% to 107% of the nominal concentration.

Mortality: There were 9 unscheduled deaths during the study. The main test article-related findings in each dog that was either found dead or euthanized *in extremis* are grouped by dose and detailed below (sponsor's table 1 and reviewer's table, below). Hypocellularity of the bone marrow was observed in 1/2 LD, 2/2 MD and 3/5 HD early decedents; femoral bone marrow was not available for assessment in 1/5 HD animals. Lymphoid depletion of lymph nodes and lymphoid tissue (Peyer's patches and/or thymus) was observed in 1/2 LD, 2/2 MD and 5/5 HD animals. A consistent effect on hematology parameters was not observed in the animals exhibiting bone marrow and/ or lymphoid tissue depletion. For example, the LD animal exhibiting panleukopenia did exhibit severe bone marrow and lymphoid tissue depletion; however, no other animal exhibiting bone marrow or lymphoid tissue depletion exhibited panleukopenia. Lung inflammation was observed in 1/2 LD, 1/2 MD, and 2/5 HD early decedents. Similar histological findings (bone marrow depletion, lymphoid depletion, and lung inflammation) were not observed to occur in a test article-related manner in animals that survived to the end of the dosing period or the end of the recovery period (see histology section below). Since only two of the early decedents were in the TK group, there were limited TK data for early decedents. LDM 5583-07 exhibited the lowest plasma concentrations of BIA 2-093 and its metabolites relative to the LDM animals assessed on PND 21; however, on PND 174, the C_{max} for BIA 2-194 (8818 ng/mL) and OXC (985

ng/mL) in this animal were approximately twice as high as the LD group mean C_{max} (BIA 2-194= 4740 ng/mL; OXC = 555 ng/mL). MDF 5574-04 exhibited plasma concentrations of BIA 2-093 and metabolites that were similar to other MDF at PND 21 and PND 174.

Text Table 1. Summary of Unscheduled Deaths

PND of Death	No. Of Doses Received	Group	Sex	Animal Number	Disposition
Toxicology Group					
61	40	2	M	5569-01[a]	Found dead
61	40	3	F	5565-05	Euthanized *in extremis*
62	40	4	M	5567-02	Found dead
62	38	4	F	5568-02	Euthanized *in extremis*
70	40	4	F	5569-04[a]	Found dead
156	118	4	F	5574-03[a]	Euthanized *in extremis*
171	150	4	M	5566-01	Euthanized *in extremis*
Toxicokinetic Group					
229	210	3	F	5574-04[a]	Euthanized *in extremis*
323	302	2	M	5583-07	Euthanized *in extremis*

[a] = Pairs of siblings included animal nos. 5569-01 and 5569-04, and animal nos. 5574-03 and 5574-04

Dose (mg/kg)	**Animal Number**	**Death (PND)**	**Clinical Signs**	**BW**	**Clinical Chemistry & Hematology**	**Histopathology**
40	5569-01	61	Thin, Dermal Atonia	-60%	WBC -80%, Monocyte -87% Lymphocyte -80% Basophils -80% Reticulocytes -46%	Panleukopenia, **Hypocellular marrow** (severe), **Lymphoid depletion** of mandibular (mild) and mesenteric (severe) lymph node, Peyer's patches (severe), Thymus (severe), **Lung inflammation** (severe)
	5583-07	302	Continuous convulsions, hypoactivity, labored respiration	+16%	N/A	None
80	5565-05	61	Hypoactivity, tremors, vocalization, impaired equilibrium	-51%	Neutrophils -18% Albumin -25% T. Protein -15% GGT +47% Cholesterol +36%	**Hypocellular marrow** (mild), **Lymphoid depletion** of mandibular (moderate) and mesenteric lymph node (moderate), Peyer's patches (severe), Thymus (not analyzed), **Lung inflammation** (mild)
	5574-04	229	Hypoactivity, tremors, impaired muscle coordination	-54%	N/A	**Hypocellular marrow** (mild-moderate), **Lymphoid depletion** of mandibular (mild) and mesenteric (mild) lymph node, Peyer's

						patches (mild), Thymus (severe)
160	5567-02	62	Hypoactivity, tremors, nystagmus, head shaking	-41%	GGT +37% Cholesterol +82%	**Hypocellular marrow** (mild), **Lymphoid depletion** of mandibular (moderate) lymph node, Peyer's patches (mild), Thymus (mild). Autolysis confounded interpretation. **Lung inflammation** (mild)
	5568-02	62	Hypoactivity Tremors, Nystagmus, Vocalization, Head tilt, Excessive salivation,	-35%	WBC -30% Platelets -29% Monocytes -20%	**Hypocellular marrow** (moderate), **Lymphoid depletion** of mandibular (moderate) and mesenteric (moderate) lymph nodes, Peyer's patches (moderate), Thymus (severe), **Lung inflammation** (severe) with hemorrhage
	5569-04	70	hypoactivity, tremors, nystagmus, vocalization, impaired equilibrium, impaired coordination	-51%	WBC -10% Neutrophils -24% Eosinophils +100% Cholesterol +12% Triglycerides +30%	**Cause of Death Lung** Inflammation (severe), **Hypocellular marrow** (mild), **Lymphoid depletion** of mandibular (mild) and mesenteric (mild) lymph nodes, Peyer's patches (moderate), Thymus (severe)
	5566-01	171	Hypoactivity, tremors, convulsions, nystagmus, head tilt, rigid muscle tone, vocalization, impaired equilibrium and muscle coordination	-38%	Lymphocytes -18% Neutrophils +40% Cholesterol +23% Triglycerides 2.2x	**Lymphoid depletion** of mandibular (minimal) and mesenteric lymph node
	5574-03	118	Hypoactivity, tremors, nystagmus, vocalization, impaired equilibrium and muscle coordination	-40%	WBC -19% Lymphocytes -26% Neutrophils -16% ALP +40% Glucose -50% GGT +78%	Femur marrow not available for assessment. **Lymphoid depletion** in thymus (moderate), **Lung Inflammation** (mild)

<u>Reviewer's Table</u>: Summary of clinical signs, BW, clinical chemistry, hematology and

histopathology findings in early decedents. % values for individual animals are relative to the mean value for the animal's respective dose group; N/A=not available (animal was in TK group); None= no test article-related findings.

Clinical Signs: Decreased activity was the only clinical sign observed to occur in more than one LD animal; continuous convulsions and impaired muscle coordination was observed in 1 LDM and 1 LDF, respectively (sponsor's table below). Test article-related clinical signs in the MD and HD groups consisted of decreased activity, hypoactivity, impaired muscle coordination, impaired equilibrium, intermittent tremors, vocalization, labored respiration, thinness, excess salivation, emesis, pale gums, and clear frothy material around muzzle. HD animals exhibited rigid muscle tone, continuous tremors, convulsions (1M #5566-01 & 3F #5570-04, #5573-04, #5582-06), alteration in respiration rate, gasping, hypothermia, and nystagmus. Generally, clinical signs resolved before the next dosing. These clinical signs were not observed during the recovery period.

Text Table 2: Selected Clinical Observations: Total Number of Occurrences/Number of Dogs

	Toxicology Animals								Toxicokinetic Animals							
	Males				Females				Males				Females			
Dose Level (mg/kg/day)	**0**	**40**	**80**	**160**	**0**	**40**	**80**	**160**	**0**	**40**	**80**	**160**	**0**	**40**	**80**	**160**
No. of Dogs	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**9**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
Tremors, Intermittent																
0.5 hour post-dosing	-	-	4/2	7/5	-	-	1/1	11/7	-	-	-	1/1	-	-	-	3/2
1 hour post-dosing	-	-	8/3	76/8	-	-	7/4	99/8	-	-	3/1	11/3	-	-	3/2	20/4
2 hours post-dosing	-	-	6/2	117/8	-	-	5/4	207/9	-	-	4/2	34/4	-	-	5/4	49/4
4 hours post-dosing	-	-	1/1	10/5	-	-	2/2	27/9	-	-	-	6/3	-	-	-	3/2
Unsch. Obs (>38-<45 minutes)*	-	-	-	-	-	-	-	1/1	-	-	-	-	-	-	-	-
Unsch. Obs (>75-<105 minutes)	-	-	-	5/3	-	-	-	13/5	-	-	-	3/2	-	-	-	3/2
Unsch. Obs (>135-<210 minutes)	-	-	-	4/3	-	-	-	8/4	-	-	-	2/2	-	-	-	-
Unsch. Obs (>270 minutes)	-	-	-	-	-	-	-	1/1	-	-	-	-	-	-	-	-
Tremors, Continuous																
0.5 hour post-dosing	-	-	-	1/1	-	-	-	-	-	-	-	-	-	-	1/1	-
1 hour post-dosing	-	-	-	5/3	-	-	1/1	1/1	-	-	-	1/1	-	-	1/1	-
2 hours post-dosing	-	-	-	13/4	-	-	-	16/4	-	-	-	-	-	-	-	2/1
4 hours post-dosing	-	-	-	1/1	-	-	-	3/1	-	-	-	-	-	-	-	1/1
Unsch. Obs (>75-<105 minutes)	-	-	-	3/1	-	-	-	3/2	-	-	-	-	-	-	-	1/1
Unsch. Obs (>135-<210 minutes)	1/1	-	-	-	-	-	-	1/1	-	-	-	-	-	-	-	-
Convulsions, Intermittent																
1 hour post-dosing	-	-	-	-	-	-	-	1/1	-	-	-	-	-	-	-	-
2 hours post-dosing	-	-	-	1/1	-	-	-	5/2	-	-	-	-	-	-	-	-
4 hours post-dosing	-	-	-	1/1	-	-	-	1/1	-	-	-	-	-	-	-	-
Unsch. Obs (>75-<105 minutes)	-	-	-	-	-	-	-	-	-	-	-	1/1	-	-	-	1/1
Unsch. Obs (>135-<210 minutes)	-	-	-	-	-	-	-	4/1	-	-	-	-	-	-	-	-

- = Not noted; Unsch. Obs = Unscheduled observations
*= >38-<45 minutes for toxicokinetic group animals

Text Table 2: Selected Clinical Observations: Total Number of Occurrences/Number of Dogs (continued)

	Toxicology Animals								Toxicokinetic Animals							
	Males				Females				Males				Females			
Dose Level (mg/kg/day)	**0**	**40**	**80**	**160**	**0**	**40**	**80**	**160**	**0**	**40**	**80**	**160**	**0**	**40**	**80**	**160**
No. of Dogs	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**9**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
Convulsions, Continuous																
1 hour post-dosing	-	-	-	2/1	-	-	-	-	-	-	-	-	-	-	-	-
2 hours post-dosing	-	-	-	2/1	-	-	-	-	-	-	-	-	-	-	-	-
Unsch. Obs (>75-<105 minutes)	-	-	-	2/1	-	-	-	-	-	-	-	-	-	-	-	-
Unsch. Obs (>270 minutes)	-	-	-	-	-	-	-	-	-	5/1	-	-	-	-	-	-
Impaired Muscle Coordination																
0.5 hour post-dosing	-	-	2/1	16/7	-	-	3/2	17/7	-	-	-	-	-	-	-	-
1 hour post-dosing	-	-	8/5	136/7	-	-	18/5	143/7	-	-	1/1	29/4	-	-	4/2	31/4
2 hours post-dosing	-	-	7/5	175/7	-	1/1	13/5	247/9	-	-	3/2	74/4	-	-	2/2	69/4
4 hours post-dosing	-	-	-	3/2	-	-	2/2	17/5	-	-	-	2/2	-	-	-	1/1
Unsch. Obs (>75-<105 minutes)	-	-	-	1/1	-	-	-	2/2	-	-	-	1/1	-	-	-	2/1
Unsch. Obs (>135-<210 minutes)	1/1	-	-	-	-	-	-	2/2	-	-	-	-	-	-	-	-
Impaired Equilibrium																
0.5 hour post-dosing	-	-	1/1	23/7	-	-	2/2	17/6	-	-	-	1/1	-	-	2/2	4/2
1 hour post-dosing	-	-	14/6	153/8	-	-	14/5	133/9	-	-	3/1	31/4	-	-	2/2	52/4
2 hours post-dosing	-	-	3/3	133/8	-	-	10/5	180/9	-	-	1/1	39/4	-	-	2/2	58/4
4 hours post-dosing	-	-	-	3/3	-	-	-	7/1	-	-	-	-	-	-	-	-
Unsch. Obs (>75-<105 minutes)	-	-	-	1/1	-	-	-	7/4	-	-	-	-	-	-	-	-
Unsch. Obs (>270 minutes)	-	-	-	1/1	-	-	-	-	-	-	-	-	-	-	-	-

- = Not noted; Unsch. Obs = Unscheduled observations

Text Table 2: Selected Clinical Observations: Total Number of Occurrences/Number of Dogs (continued)

	Toxicology Animals								Toxicokinetic Animals							
	Males				Females				Males				Females			
Dose Level (mg/kg/day)	**0**	**40**	**80**	**160**	**0**	**40**	**80**	**160**	**0**	**40**	**80**	**160**	**0**	**40**	**80**	**160**
No. of Dogs	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**9**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
Hypoactivity																
0.5 hour post-dosing	-	-	-	3/3	-	-	2/1	13/6	-	-	-	2/1	-	-	-	1/1
1 hour post-dosing	-	-	4/2	72/8	-	-	4/2	114/9	-	-	1/1	13/3	-	-	-	21/3
2 hours post-dosing	-	-	4/3	154/8	-	-	3/2	244/9	-	-	-	45/4	-	-	-	50/4
4 hours post-dosing	-	-	1/1	19/7	-	-	1/1	24/9	-	-	-	7/4	-	-	1/1	6/3
Unsch. Obs (>75-<105 minutes)	-	-	2/1	10/5	-	-	-	12/5	-	-	-	3/1	-	-	-	1/1
Unsch. Obs (>135-<210 minutes)	-	-	-	6/4	-	-	1/1	6/6	-	-	-	-	-	-	-	-
Unsch. Obs (>270 minutes)	-	-	-	-	-	-	-	2/2	-	-	-	-	-	-	-	-
Decreased Activity																
Prior to dosing	-	1/1	-	1/1	-	-	1/1	-	-	-	-	-	-	-	-	-
0.5 hour post-dosing	-	3/2	7/4	35/8	-	1/1	6/4	39/8	-	-	2/2	8/3	-	-	-	9/3
1 hour post-dosing	-	7/2	41/8	203/7	-	2/2	43/7	169/8	-	-	4/3	46/4	-	-	16/4	80/4
2 hours post-dosing	-	-	18/7	164/8	-	-	17/7	201/8	-	-	9/4	60/4	-	-	10/4	91/4
4 hours post-dosing	-	1/1	-	7/5	-	-	-	24/7	-	-	-	1/1	-	-	3/1	-
Unsch. Obs (>270 minutes)	-	-	-	-	-	-	1/1	6/3	-	-	-	-	-	-	-	-
Vocalization																
0.5 hour post-dosing	-	-	-	-	-	-	1/1	4/2	-	-	-	1/1	-	-	-	-
1 hour post-dosing	-	-	1/1	26/6	-	-	1/1	19/6	-	-	-	4/3	-	-	-	4/2
2 hours post-dosing	-	-	-	46/5	-	-	1/1	84/7	-	-	-	15/3	-	-	-	32/3
4 hours post-dosing	-	-	-	1/1	-	-	-	4/3	-	-	-	1/1	-	-	-	1/1
Unsch. Obs (>0-<22 minutes)	-	-	-	1/1	-	-	-	-	-	-	-	-	-	-	-	-
Unsch. Obs (>38-<45 minutes)	-	-	-	-	-	-	-	1/1	-	-	-	-	-	-	-	-
Unsch. Obs (>75-<105 minutes)	-	-	1/1	12/4	-	-	-	15/4	-	-	-	3/1	-	-	-	5/1

- = Not noted; Unsch. Obs = Unscheduled observations

Text Table 2: Selected Clinical Observations: Total Number of Occurrences/Number of Dogs (continued)

	Toxicology Animals								Toxicokinetic Animals							
	Males				Females				Males				Females			
Dose Level (mg/kg/day)	0	40	80	160	0	40	80	160	0	40	80	160	0	40	80	160
No. of Dogs	8	8	8	8	8	8	8	9	4	4	4	4	4	4	4	4
Vocalization (continued)																
Unsch. Obs (>135-<210 minutes)	-	-	-	4/4	-	-	-	9/5	-	-	-	-	-	-	-	-
Unsch. Obs (>270 minutes)	-	-	-	-	-	-	-	1/1	-	-	-	-	-	-	-	-
Rigid Muscle Tone																
1 hour post-dosing	-	-	-	1/1	-	-	-	2/2	-	-	-	2/2	-	-	-	2/2
2 hours post-dosing	-	-	-	14/5	-	-	-	52/7	-	-	-	7/3	-	-	-	10/3
4 hours post-dosing	-	-	-	-	-	-	-	4/2	-	-	-	-	-	-	-	2/1
Unsch. Obs (>75-<105 minutes)	-	-	-	1/1	-	-	-	4/2	-	-	-	1/1	-	-	-	-
Unsch. Obs (>135-<210 minutes)	-	-	-	-	-	-	-	5/2	-	-	-	-	-	-	-	-
Labored Respiration																
Prior to dosing	-	-	-	7/1	-	-	-	-	-	-	-	-	-	-	-	-
0.5 hour post-dosing	-	-	-	5/2	-	-	-	-	-	-	-	-	-	-	-	-
1 hour post-dosing	-	-	-	7/1	-	-	-	-	-	-	-	-	-	-	-	-
2 hours post-dosing	-	-	-	5/1	-	-	-	2/2	-	-	-	-	-	-	-	-
4 hours post-dosing	-	-	-	3/1	-	-	-	-	-	-	-	-	-	-	-	-
Unsch. Obs (>0-<22 minutes)	-	-	1/1	-	-	-	-	-	-	-	-	-	-	-	-	-
Unsch. Obs (>270 minutes)	-	-	-	-	-	-	-	-	-	1/1	-	-	-	-	-	-
Gasping																
Unsch. Obs (>0-<22 minutes)	-	-	-	1/1	-	-	-	-	-	-	-	-	-	-	-	-
Respiration Rate Decreased																
2 hours post-dosing	-	-	-	-	-	-	-	1/1	-	-	-	-	-	-	-	-
Respiration Rate Increased																
2 hours post-dosing	-	-	-	1/1	-	-	-	1/1	-	-	-	1/1	-	-	-	1/1
Unsch. Obs (>75-<105 minutes)	-	-	-	-	-	-	-	-	-	-	-	1/1	-	-	-	2/1
Unsch. Obs (>135-<210 minutes)	-	-	-	-	-	-	-	1/1	-	-	-	1/1	-	-	-	-

- = Not noted; Unsch. Obs = Unscheduled observations

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Text Table 2: Selected Clinical Observations: Total Number of Occurrences/Number of Dogs (continued)

	Toxicology Animals								Toxicokinetic Animals							
	Males				Females				Males				Females			
Dose Level (mg/kg/day)	0	40	80	160	0	40	80	160	0	40	80	160	0	40	80	160
No. of Dogs	8	8	8	8	8	8	8	9	4	4	4	4	4	4	4	4
Injected Sclera of the Right/Left Eye (continued)																
4 hours post-dosing	- 1/1	- -	4/1 4/1	1/1 1/1	6/2 4/3	2/2 1/1	2/1 2/1	6/2 5/2	1/1 -	1/1 -	- -	- -	1/1 1/1	5/3 2/2	- -	- 2/1
Nystagmus																
0.5 hour post-dosing	-	-	-	-	-	-	-	1/1	-	-	-	-	-	-	-	-
1 hour post-dosing	-	-	-	7/2	-	-	-	5/3	-	-	-	1/1	-	-	-	2/1
2 hours post-dosing	-	-	-	16/4	-	-	-	24/6	-	-	-	4/2	-	-	-	4/2
4 hours post-dosing	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1/1
Unsch. Obs (>75-<105 minutes)	-	-	-	2/1	-	-	-	3/2	-	-	-	3/2	-	-	-	-
Unsch. Obs (>135-<210 minutes)	-	-	-	2/1	-	-	-	-	-	-	-	-	-	-	-	-
Unsch. Obs (>270 minutes)	-	-	-	-	-	-	-	1/1	-	-	-	-	-	-	-	-
Excess Salivation																
Prior to Dosing	-	-	-	-	-	-	-	4/3	-	-	-	-	-	-	-	-
0.5 hour post-dosing	-	-	-	11/3	-	-	-	18/3	-	-	-	-	-	-	-	4/2
1 hour post-dosing	-	-	-	2/2	-	-	-	3/2	-	-	-	1/1	-	-	-	-
2 hours post-dosing	-	-	-	5/4	-	-	-	12/3	-	-	-	5/2	-	-	-	2/2
4 hours post-dosing	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1/1
Unsch. Obs (>0-<22 minutes)	-	-	-	5/2	-	-	-	7/2	-	-	-	-	-	-	-	-
Unsch. Obs (>75-<105 minutes)	-	-	-	-	-	-	-	-	-	-	-	2/1	-	-	-	-

Body Weight & Food Consumption: There was no test article-related effect observed in animals that survived to the end of the dosing period.

Neurobehavioral Assessment: Assessments consisted of home cage, open field, and

functional (table top) observations on PND 47, 80, 174, 328, and 384. The sponsor's lists below indicate the tests performed for each assessment.

5.5.1. HOME CAGE OBSERVATIONS*

General posture	Lacrimation
General demeanor	Palpebral (eyelid) closure
Head posture	Body weight
Convulsions/tremors	Excreta**
Salivation	Emesis**

* - For the home cage observations for PND 47 and 80, animals were removed from the dam and/or respective siblings and placed individually into a standard arena for observations.

** - These parameters were not evaluated from F_1 animals at the PND 47 and 80 home cage observations since the animals were group-housed.

5.5.2. OPEN FIELD OBSERVATIONS

Time to first step (seconds)	Behavior
Gait	

5.6. FUNCTIONAL OBSERVATIONS AND MEASUREMENTS (TABLE TOP)

Respiration rate	Patellar reflex
Respiration pattern	Pinch reflex
Perineal reflex	Proprioceptive positioning
Rectal temperature	Posterior extensor thrust
Blood pressure and heart rate (Surgivet device was used for blood pressure and heart rate measurements)*	Wheel barrowing
	Hemistanding/hemiwalking
	Capillary refill time
Cliff avoidance	Jaw and tongue examination
Pinna reflex	Gag reflex
Pupillary reflex	Auditory response
Pupillary size	Menace reaction
Nystagmus	Femur length (right femur)
Palpebral reflex	
Righting reflex	
Triceps reflex	

* - Blood pressure and heart rate were not measured from F_1 animals at the PND 47 evaluations. These parameters were not evaluated because of the inherent variability associated with these types of data collected in young animals. This variability is primarily attributed to the physical limitation of measuring these parameters from the size of animals at the indicated age.

A) Home Cage Assessment: One MDF exhibited small muscle tremors on PND 80. There were no other test article-related findings in the home cage assessments performed.

B) Open Field/ Table Top Assessment: On PND 47, pinna reflex and perineal reflex was absent in 1/8 CM, 5/8 LDM, 4/8 MDM, and 3/9 HDM. On PND 174, pinna reflex was absent in 1/8 CM, 1/7 LDM, 6/8 MDM, and 4/6 HDM. There were no

test article-related findings observed during the recovery period.

Hematology & Clinical Chemistry: Hematology and clinical chemistry findings in early decedents are discussed above in the section on mortality. When assessed on PND 49, 81, 175, 329, and 385, there were no test article-related hematology findings in animals that survived to the end of the dosing period or the end of the recovery period.

Clinical chemistry was assessed on PND 49, 81, 175, 329, and 385. Alterations related to the test article were observed in ALP, GGT, serum cholesterol, and triglycerides (sponsor's tables, below). A test article-related increase in cholesterol was observed in LD, MD, and HD males and females on PND 49, 81, 175, and 329. Triglycerides were elevated in LD, MD, and HD males and HD females (PND 175 and 329). The increase in cholesterol and triglycerides was not observed in males at the end of the recovery period; cholesterol was elevated in LD and HD females by 30-40%, and triglycerides were elevated in LD animals at the end of the recovery period. ALP was increased in all male dose groups on PND 49, 81, 175, and 329 and female dose groups on day 329. GGT was increased in all male (PND 81 and PND 329) and female (PND 49, 81, 175, 385) dose groups.

ALP (Males and Females)

ANALYSIS	GROUP:	MALES 0 MG/KG/DAY	40 MG/KG/DAY	80 MG/KG/DAY	160 MG/KG/DAY
ALKALINEPHOS'TSE (U/L)					
PND 49	MEAN	134.	150.	155.	158.
	% DIFFERENCE		11.9	15.7	17.9
	S.D.	14.6	30.3	17.8	28.7
	S.E.	5.2	10.7	6.3	10.1
	N	8	8	8	8
PND 81	MEAN	172.	210.	235.**	208.
	% DIFFERENCE		22.1	36.6	20.9
	S.D.	22.2	32.3	41.7	43.2
	S.E.	7.9	12.2	14.7	16.3
	N	8	7	8	7
PND 175	MEAN	110.	138.*	140.*	135.
	% DIFFERENCE		25.5	27.3	22.7
	S.D.	9.8	24.3	23.4	14.9
	S.E.	3.5	9.2	8.3	6.1
	N	8	7	8	6
PND 329	MEAN	54.	72.	70.	68.
	% DIFFERENCE		33.3	29.6	25.9
	S.D.	10.4	28.4	17.6	22.4
	S.E.	3.7	10.8	6.2	9.1
	N	8	7	8	6
ALKALINEPHOS'TSE (U/L)					
PND 385	MEAN	70.	52.	50.	40.
	% DIFFERENCE		-25.7	-28.6	-42.9
	S.D.	25.8	13.4	14.2	3.5
	S.E.	14.9	9.5	8.2	2.5
	N	3	2	3	2

mg/dL - MILLIGRAMS/DECILITER, U/L - INTERNATIONAL UNIT/LITER, g/dL - GRAMS/DECILITER, mEq/L - MILLIEQUIVALENTS/LITER

* - Significantly different from the control group at 0.05 using Dunnett's test
** - Significantly different from the control group at 0.01 using Dunnett's test

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ANALYSIS GROUP:		FEMALES 0 MG/KG/DAY	40 MG/KG/DAY	80 MG/KG/DAY	160 MG/KG/DAY
ALKALINEPHOS'TSE (U/L)					
PND 49	MEAN	143.	146.	146.	161.
	% DIFFERENCE		2.1	2.1	12.6
	S.D.	25.3	35.9	30.8	43.8
	S.E.	8.9	12.7	10.9	14.6
	N	8	8	8	9
PND 81	MEAN	183.	206.	197.	187.
	% DIFFERENCE		12.6	7.7	2.2
	S.D.	27.6	57.0	33.4	25.0
	S.E.	9.8	20.2	12.6	9.5
	N	8	8	7	7
PND 175	MEAN	112.	128.	124.	128.
	% DIFFERENCE		14.3	10.7	14.3
	S.D.	13.2	22.1	37.9	19.0
	S.E.	4.7	7.8	14.3	7.7
	N	8	8	7	6
PND 329	MEAN	49.	62.	87.	77.
	% DIFFERENCE		26.5	77.6	57.1
	S.D.	13.8	17.7	39.7	29.4
	S.E.	4.9	6.2	15.0	12.0
	N	8	8	7	6
ALKALINEPHOS'TSE (U/L)					
PND 385	MEAN	39.	62.	58.	36.
	% DIFFERENCE		59.0	48.7	-7.7
	S.D.	8.6	32.5	18.4	4.2
	S.E.	5.0	18.8	13.0	3.0
	N	3	3	2	2

mg/dL - MILLIGRAMS/DECILITER, U/L - INTERNATIONAL UNIT/LITER, g/dL - GRAMS/DECILITER, mEq/L - MILLIEQUIVALENTS/LITER

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GGT (Males and Females)

ANALYSIS GROUP:		MALES 0 MG/KG/DAY	40 MG/KG/DAY	80 MG/KG/DAY	160 MG/KG/DAY
GLUTAMYLTRANSFER (U/L)					
PND 49	MEAN	1.5	0.9	1.5	2.4
	% DIFFERENCE		-40.0	0.0	60.0
	S.D.	0.83	0.99	0.87	0.93
	S.E.	0.30	0.35	0.31	0.33
	N	8	8	8	8
PND 81	MEAN	2.9	3.6	3.4	3.8
	% DIFFERENCE		24.1	17.2	31.0
	S.D.	0.84	0.56	0.77	1.02
	S.E.	0.30	0.21	0.27	0.39
	N	8	7	8	7
PND 175	MEAN	2.1	2.4	1.9	2.7
	% DIFFERENCE		14.3	-9.5	28.6
	S.D.	0.96	0.52	1.18	1.48
	S.E.	0.34	0.20	0.42	0.60
	N	8	7	8	6
PND 329	MEAN	1.1	1.7	2.3*	2.2*
	% DIFFERENCE		54.5	109.1	100.0
	S.D.	0.93	0.90	0.76	0.47
	S.E.	0.33	0.34	0.27	0.19
	N	8	7	8	6
GLUTAMYLTRANSFER (U/L)					
PND 385	MEAN	0.9	1.9	2.1	1.7
	% DIFFERENCE		111.1	133.3	88.9
	S.D.	1.19	1.70	1.37	0.28
	S.E.	0.69	1.20	0.79	0.20
	N	3	2	3	2

mg/dL - MILLIGRAMS/DECILITER, U/L - INTERNATIONAL UNIT/LITER, g/dL - GRAMS/DECILITER, mEq/L - MILLIEQUIVALENTS/LITER

* - Significantly different from the control group at 0.05 using Dunnett's test

FEMALES

ANALYSIS GROUP:	0 MG/KG/DAY	40 MG/KG/DAY	80 MG/KG/DAY	160 MG/KG/DAY
GLUTAMYLTRANSFER (U/L)				
PND 49 MEAN	1.1	1.4	1.9	2.5**
% DIFFERENCE		27.3	72.7	127.3
S.D.	0.72	0.74	0.74	1.03
S.E.	0.25	0.26	0.26	0.34
N	8	8	8	9
PND 81 MEAN	2.9	3.4	3.7	3.8
% DIFFERENCE		17.2	27.6	31.0
S.D.	0.52	0.60	1.11	1.48
S.E.	0.18	0.21	0.42	0.56
N	8	8	7	7
PND 175 MEAN	1.4	2.0	2.2	2.5
% DIFFERENCE		42.9	57.1	78.6
S.D.	0.54	1.43	0.64	0.79
S.E.	0.19	0.51	0.24	0.32
N	8	8	7	6
PND 329 MEAN	1.2	1.4	2.2	1.5
% DIFFERENCE		16.7	83.3	25.0
S.D.	1.10	0.86	1.02	1.19
S.E.	0.39	0.30	0.38	0.49
N	8	8	7	6
GLUTAMYLTRANSFER (U/L)				
PND 385 MEAN	1.1	2.4	3.8	1.7
% DIFFERENCE		118.2	245.5	54.5
S.D.	1.31	1.46	2.12	0.71
S.E.	0.75	0.84	1.50	0.50
N	3	3	2	2

mg/dL = MILLIGRAMS/DECILITER, U/L = INTERNATIONAL UNIT/LITER, g/dL = GRAMS/DECILITER, mEq/L = MILLIEQUIVALENTS/LITER

** = Significantly different from the control group at 0.01 using Dunnett's test

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Cholesterol (Males and Females)

MALES

ANALYSIS GROUP:	0 MG/KG/DAY	40 MG/KG/DAY	80 MG/KG/DAY	160 MG/KG/DAY
CHOLESTEROL (mg/dL)				
PND 49 MEAN	220.	283.	292.	308.
% DIFFERENCE		28.6	32.7	40.0
S.D.	82.7	62.7	105.5	116.7
S.E.	29.3	22.2	37.3	41.3
N	8	8	8	8
PND 81 MEAN	126.	149.	146.	148.
% DIFFERENCE		18.3	15.9	17.5
S.D.	21.8	21.0	29.8	35.9
S.E.	7.7	7.9	10.6	13.6
N	8	7	8	7
PND 175 MEAN	135.	175.	190.**	202.**
% DIFFERENCE		29.6	40.7	49.6
S.D.	23.7	9.5	36.3	50.9
S.E.	8.4	3.6	12.8	20.8
N	8	7	8	6
PND 329 MEAN	123.	170.*	207.**	202.**
% DIFFERENCE		38.2	68.3	64.2
S.D.	24.4	22.9	37.4	23.1
S.E.	8.6	8.7	13.2	9.4
N	8	7	8	6
CHOLESTEROL (mg/dL)				
PND 385 MEAN	152.	140.	155.	133.
% DIFFERENCE		-7.9	2.0	-12.5
S.D.	23.4	12.0	1.7	8.5
S.E.	13.5	8.5	1.0	6.0
N	3	2	3	2

mg/dL = MILLIGRAMS/DECILITER, U/L = INTERNATIONAL UNIT/LITER, g/dL = GRAMS/DECILITER, mEq/L = MILLIEQUIVALENTS/LITER

* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test

ANALYSIS	GROUP:	FEMALES 0 MG/KG/DAY	40 MG/KG/DAY	80 MG/KG/DAY	160 MG/KG/DAY
CHOLESTEROL (mg/dL)					
PND 49	MEAN	238.	323.	248.	334.
	% DIFFERENCE		35.7	4.2	40.3
	S.D.	88.3	148.1	45.7	129.2
	S.E.	31.2	52.4	16.2	43.1
	N	8	8	8	9
PND 81	MEAN	126.	147.	140.	169.**
	% DIFFERENCE		16.7	11.1	34.1
	S.D.	19.2	24.5	24.7	31.2
	S.E.	6.8	8.7	9.3	11.8
	N	8	8	7	7
PND 175	MEAN	140.	167.	174.*	201.**
	% DIFFERENCE		19.3	24.3	43.6
	S.D.	10.1	27.8	32.7	20.2
	S.E.	3.6	9.8	12.3	8.2
	N	8	8	7	6
PND 329	MEAN	148.	182.	203.*	239.**
	% DIFFERENCE		23.0	37.2	61.5
	S.D.	24.1	20.5	53.2	38.9
	S.E.	8.5	7.3	20.1	15.9
	N	8	8	7	6
CHOLESTEROL (mg/dL)					
PND 385	MEAN	136.	180.	139.	193.
	% DIFFERENCE		32.4	2.2	41.9
	S.D.	5.1	39.3	2.8	54.4
	S.E.	3.0	22.7	2.0	38.5
	N	3	3	2	2

mg/dL - MILLIGRAMS/DECILITER, U/L - INTERNATIONAL UNIT/LITER, g/dL - GRAMS/DECILITER, mEq/L - MILLIEQUIVALENTS/LITER

* - Significantly different from the control group at 0.05 using Dunnett's test
** - Significantly different from the control group at 0.01 using Dunnett's test

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Triglycerides (Males and Females)

ANALYSIS	GROUP:	MALES 0 MG/KG/DAY	40 MG/KG/DAY	80 MG/KG/DAY	160 MG/KG/DAY
TRIGLYCERIDE (mg/dL)					
PND 49	MEAN	80.	102.	120.	98.
	% DIFFERENCE		27.5	50.0	22.5
	S.D.	13.9	23.9	60.8	54.3
	S.E.	4.9	8.5	21.5	19.2
	N	8	8	8	8
PND 81	MEAN	46.	58.	50.	57.
	% DIFFERENCE		26.1	8.7	23.9
	S.D.	15.9	16.9	15.4	20.3
	S.E.	5.6	6.4	5.4	7.7
	N	8	7	8	7
PND 175	MEAN	22.	42.**	46.**	41.**
	% DIFFERENCE		90.9	109.1	86.4
	S.D.	6.5	9.8	11.3	12.1
	S.E.	2.3	3.7	4.0	4.9
	N	8	7	8	6
PND 329	MEAN	20.	37.**	39.**	39.**
	% DIFFERENCE		85.0	95.0	95.0
	S.D.	6.2	11.1	8.9	8.2
	S.E.	2.2	4.2	3.2	3.4
	N	8	7	8	6
TRIGLYCERIDE (mg/dL)					
PND 385	MEAN	40.	26.	31.	38.
	% DIFFERENCE		-35.0	-22.5	-5.0
	S.D.	9.5	11.3	7.0	8.5
	S.E.	5.5	8.0	4.1	6.0
	N	3	2	3	2

mg/dL - MILLIGRAMS/DECILITER, U/L - INTERNATIONAL UNIT/LITER, g/dL - GRAMS/DECILITER, mEq/L - MILLIEQUIVALENTS/LITER

** - Significantly different from the control group at 0.01 using Dunnett's test

ANALYSIS	GROUP:	FEMALES 0 MG/KG/DAY	40 MG/KG/DAY	80 MG/KG/DAY	160 MG/KG/DAY
TRIGLYCERIDE (mg/dL)					
PND 49	MEAN	84.	87.	76.	97.
	% DIFFERENCE		3.6	-9.5	15.5
	S.D.	25.2	14.4	17.0	35.2
	S.E.	8.9	5.1	6.0	11.7
	N	8	8	8	9
PND 81	MEAN	50.	50.	54.	59.
	% DIFFERENCE		0.0	8.0	18.0
	S.D.	17.8	7.7	13.0	21.6
	S.E.	6.3	2.7	4.9	8.2
	N	8	8	7	7
PND 175	MEAN	27.	31.	36.	46.**
	% DIFFERENCE		14.8	33.3	70.4
	S.D.	5.8	5.8	11.4	13.0
	S.E.	2.1	2.0	4.3	5.3
	N	8	8	7	6
PND 329	MEAN	30.	38.	42.	48.
	% DIFFERENCE		26.7	40.0	60.0
	S.D.	11.7	19.9	13.9	9.2
	S.E.	4.2	7.0	5.3	3.8
	N	8	8	7	6
TRIGLYCERIDE (mg/dL)					
PND 385	MEAN	23.	34.	25.	23.
	% DIFFERENCE		47.8	8.7	0.0
	S.D.	2.0	9.6	2.1	1.4
	S.E.	1.2	5.5	1.5	1.0
	N	3	3	2	2

mg/dL - MILLIGRAMS/DECILITER, U/L - INTERNATIONAL UNIT/LITER, g/dL - GRAMS/DECILITER, mEq/L - MILLIEQUIVALENTS/LITER

** - Significantly different from the control group at 0.01 using Dunnett's test

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Urinalysis: There were no test article-related findings when assessed on PND 49, 81, 175, 329, or 385.

Ophthalmic Examination: When assessed during the last week of the dosing period and during the last week of the recovery period, there were no test article-related findings.

EEG: There were no test article-related findings on heart rate or RR, QRS, PR, QT, or QTc (Van de Water correction) intervals when assessed at PND 322 and PND 378.

Gross Pathology: One HDF (5582-06) euthanized at the end of the dosing period exhibited hydrocephaly and increased cavitation of the lateral and third ventricles. There were no other test article-related findings.

Organ Weights: Absolute liver weight and weight relative to BW were increased at the end of the dosing period in all male dose groups (sponsor's table, below); a similar finding was not observed in females. Absolute prostate weight and weight relative to BW were lower in HDM than in control. Absolute thymus weight and weight relative to BW was increased in HDM. Brain weight, relative to BW, was increased in all female dose groups. Absolute ovary, thymus, and uterus weights were decreased in all female dose groups. Decreased prostate weight was observed at the end of the recovery period (sponsor's table below).

Males (End of Dosing Period)

PROJECT NO. (b) (A)-682002M
SPONSOR:BIAL-PORTELA & CA

TABLE 36 (PND 329 PRIMARY NECROPSY)
A 10-MONTH ORAL STUDY OF BIA 2-093 IN JUVENILE DOGS
SUMMARY OF ORGAN WEIGHTS AND RELATIVE ORGAN WEIGHTS

GROUP:	MALES 0 MG/KG/DAY	40 MG/KG/DAY	80 MG/KG/DAY	160 MG/KG/DAY
LIVER (G)				
MEAN	244.69	354.53**	305.45	323.22*
% DIFFERENCE		44.9	24.8	32.1
S.D.	29.153	66.624	25.275	32.357
S.E.	13.037	29.795	11.303	16.179
N	5	5	5	4
LIVER (G/100 G FINAL BODY WEIGHT)				
MEAN	2.398	2.859	3.250**	3.266*
% DIFFERENCE		19.2	35.5	36.2
S.D.	0.2564	0.2423	0.4205	0.5669
S.E.	0.1147	0.1084	0.1880	0.2835
N	5	5	5	4
PROSTATE (G)				
MEAN	6.52	7.06	6.12	4.53
% DIFFERENCE		8.3	-6.1	-30.5
S.D.	3.036	3.401	2.979	1.956
S.E.	1.358	1.521	1.332	0.978
N	5	5	5	4
PROSTATE (G/100 G FINAL BODY WEIGHT)				
MEAN	0.064	0.058	0.065	0.046
% DIFFERENCE		-9.4	1.6	-28.1
S.D.	0.0333	0.0275	0.0308	0.0203
S.E.	0.0149	0.0123	0.0138	0.0101
N	5	5	5	4
THYMUS (G)				
MEAN	6.81	8.28	6.98	9.11
% DIFFERENCE		21.6	2.5	33.8
S.D.	2.978	5.411	2.692	2.805
S.E.	1.332	2.420	1.204	1.403
N	5	5	5	4
THYMUS (G/100 G FINAL BODY WEIGHT)				
MEAN	0.065	0.063	0.074	0.092
% DIFFERENCE		-3.1	13.8	41.5
S.D.	0.0250	0.0344	0.0264	0.0308
S.E.	0.0112	0.0154	0.0118	0.0154
N	5	5	5	4

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* - Significantly different from the control group at 0.05 using Dunnett's test
** - Significantly different from the control group at 0.01 using Dunnett's test

Females (End of Dosing Period)

GROUP:	FEMALES 0 MG/KG/DAY	40 MG/KG/DAY	80 MG/KG/DAY	160 MG/KG/DAY
BRAIN (G/100 G FINAL BODY WEIGHT)				
MEAN	0.777	0.848	0.916	1.078
% DIFFERENCE		9.1	17.9	38.7
S.D.	0.0629	0.1281	0.2222	0.2712
S.E.	0.0281	0.0573	0.0993	0.1356
N	5	5	5	4
OVARIES (G)				
MEAN	1.5489	1.1325	1.1936	0.8981
% DIFFERENCE		-26.9	-22.9	-42.0
S.D.	0.58961	0.91596	0.49878	0.58431
S.E.	0.26368	0.40963	0.22306	0.29216
N	5	5	5	4
OVARIES (G/100 G FINAL BODY WEIGHT)				
MEAN	0.016	0.012	0.014	0.012
% DIFFERENCE		-25.0	-12.5	-25.0
S.D.	0.0066	0.0087	0.0062	0.0056
S.E.	0.0030	0.0039	0.0028	0.0028
N	5	5	5	4
THYMUS (G)				
MEAN	10.43	5.35*	5.44*	6.39
% DIFFERENCE		-48.7	-47.8	-38.7
S.D.	2.344	2.573	2.836	2.568
S.E.	1.048	1.151	1.268	1.284
N	5	5	5	4
UTERUS/CERVIX (G)				
MEAN	11.89	5.85	9.89	5.06
% DIFFERENCE		-50.8	-16.8	-57.4
S.D.	7.439	6.883	10.520	6.707
S.E.	3.327	3.078	4.705	3.353
N	5	5	5	4
UTERUS/CERVIX (G/100 G FINAL BODY WEIGHT)				
MEAN	0.124	0.063	0.110	0.061
% DIFFERENCE		-49.2	-11.3	-50.8
S.D.	0.0806	0.0694	0.1107	0.0726
S.E.	0.0360	0.0310	0.0495	0.0363
N	5	5	5	4

Males (End of Recovery Period)

PROJECT NO. (b) (4) 682002M
SPONSOR: BIAL-PORTELA & CA

TABLE 37 (PND 385 RECOVERY NECROPSY)
A 10-MONTH ORAL STUDY OF BIA 2-093 IN JUVENILE DOGS
SUMMARY OF ORGAN WEIGHTS AND RELATIVE ORGAN WEIGHTS

PAGE 7

GROUP:	MALES 0 MG/KG/DAY	40 MG/KG/DAY	80 MG/KG/DAY	160 MG/KG/DAY
PROSTATE (G)				
MEAN	7.26	7.05	7.01	6.28
% DIFFERENCE		-2.9	-3.4	-13.5
S.D.	0.756	1.372	0.835	1.110
S.E.	0.437	0.970	0.482	0.785
N	3	2	3	2
PROSTATE (G/100 G FINAL BODY WEIGHT)				
MEAN	0.074	0.063	0.059	0.053
% DIFFERENCE		-14.9	-20.3	-28.4
S.D.	0.0171	0.0198	0.0169	0.0057
S.E.	0.0099	0.0140	0.0098	0.0040
N	3	2	3	2

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Bone Densitometry: Femur and lumbar vertebrae (L3-5) were assessed for bone mineral content, area, density, and length (femur only) at the end of the dosing and recovery periods. There were no test article-related effects observed in males (sponsor's tables, below). In females, a dose-dependent decrease in bone mineral content (lumbar= 10-28%; femur= 5-25%), bone area (lumbar= 3-13%; femur= 4-14%), and bone mineral density (lumbar= 8-17%; femur (MD and HD only) = 7-13%) was observed at the end of the dosing period but not at the end of the recovery period (sponsor's tables, below). Femur length was reduced by 7% in HDFs; there was no test article-related effect on femur length in LDF or MDF.

Male Lumbar Vertebrae 3-5

Table 2. Summary DXA Scan Results of the LV3-5

		Terminal			Recovery		
Group	Data	Bone Mineral Content	Bone Area	Bone Mineral Density	Bone Mineral Content	Bone Area	Bone Mineral Density
		g	cm^2	g/cm^2	g	cm^2	g/cm^2
Vehicle Group	Mean	10.347	14.603	0.702	9.985	13.840	0.72
Male	SD	2.563	1.674	0.091	2.615	2.417	0.10
0 mg/kg/day	n	5	5	5	3	3	3
	Stat	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
BIA 2-093	Mean	12.355	16.170	0.761	10.441	14.589	0.72
Male	SD	2.189	1.767	0.065	0.066	0.448	0.03
40 mg/kg/day	n	5	5	5	2	2	2
	Stat	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
BIA 2-093	Mean	9.376	13.747	0.682	10.782	15.614	0.69
Male	SD	0.992	0.534	0.065	1.486	1.318	0.04
80 mg/kg/day	n	5	5	5	3	3	3
	Stat	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
BIA 2-093	Mean	9.880	14.509	0.680	11.788	15.173	0.78
Male	SD	1.186	0.963	0.048	0.115	0.628	0.02
160 mg/kg/day	n	4	4	4	2	2	2
	Stat	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Male Femur

Table 3. Summary DXA Scan Results at the Whole Femur

		Terminal				Recovery			
Group	Data	Bone Mineral Content	Bone Area	Bone Mineral Density	Femur Length	Bone Mineral Content	Bone Area	Bone Mineral Density	Femur Length
		g	cm^2	g/cm^2	cm	g	cm^2	g/cm^2	cm
Vehicle Group	Mean	12.982	25.630	0.501	13.770	11.651	23.786	0.49	12.75
Male	SD	3.170	2.916	0.064	1.188	2.215	3.382	0.04	0.65
0 mg/kg/day	n	5	5	5	5	3	3	3	3
	Stat	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
BIA 2-093	Mean	15.294	28.378	0.536	14.250	13.479	26.382	0.51	13.65
Male	SD	2.715	2.934	0.043	1.012	1.034	0.441	0.03	0.00
40 mg/kg/day	n	5	5	5	5	2	2	2	2
	Stat	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
BIA 2-093	Mean	11.316	24.214	0.467	13.110	13.611	26.870	0.50	13.95
Male	SD	0.870	0.586	0.032	0.481	2.672	2.310	0.06	0.65
80 mg/kg/day	n	5	5	5	5	3	3	3	3
	Stat	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
BIA 2-093	Mean	12.102	25.321	0.477	13.538	14.619	27.515	0.53	14.18
Male	SD	1.239	1.354	0.033	0.708	0.388	0.006	0.01	0.11
160 mg/kg/day	n	4	4	4	4	2	2	2	2
	Stat	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Female Lumbar Vertebrae 3-5

		Terminal			Recovery		
Group	Data	Bone Mineral Content	Bone Area	Bone Mineral Density	Bone Mineral Content	Bone Area	Bone Mineral Density
		g	cm^2	g/cm^2	g	cm^2	g/cm^2
Vehicle Group	Mean	9.750	13.871	0.701	8.740	12.667	0.69
Female	SD	1.479	1.113	0.073	0.428	0.990	0.02
0 mg/kg/day	n	5	5	5	3	3	3
	Stat	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
BIA 2-093	Mean	8.782	13.583	0.645	7.159	11.620	0.61
Female	SD	1.015	0.965	0.036	1.857	2.182	0.08
40 mg/kg/day	n	5	5	5	3	3	3
	Stat	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
BIA 2-093	Mean	8.499	13.242	0.636	7.784	12.680	0.61
Female	SD	1.999	1.858	0.067	0.313	0.695	0.01
80 mg/kg/day	n	5	5	5	2	2	2
	Stat	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
BIA 2-093	Mean	7.101	12.201	0.582	9.468	13.115	0.72
Female	SD	0.591	0.668	0.029	0.167	0.016	0.01
160 mg/kg/day	n	4	4	4	2	2	2
	Stat	*	n.s.	*	n.s.	n.s.	n.s.

n.a. = not applicable n.s. = not significant
* $p < 0.05$ when compared to vehicle-treated group of the same sex using Dunnett's test

Female Femur

		Terminal				Recovery			
Group	Data	Bone Mineral Content	Bone Area	Bone Mineral Density	Femur Length	Bone Mineral Content	Bone Area	Bone Mineral Density	Femur Length
Vehicle Group	Mean	11.490	24.401	0.470	13.020	10.028	21.954	0.46	12.60
Female	SD	1.589	1.935	0.034	0.479	1.495	2.035	0.03	0.75
0 mg/kg/day	n	5	5	5	5	3	3	3	3
	Stat	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
BIA 2-093	Mean	10.964	23.464	0.466	13.200	9.236	20.819	0.44	12.20
Female	SD	1.380	2.139	0.021	0.600	2.136	4.115	0.05	0.83
40 mg/kg/day	n	5	5	5	5	3	3	3	3
	Stat	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
BIA 2-093	Mean	10.262	23.175	0.441	13.050	9.778	22.419	0.43	12.53
Female	SD	1.643	1.961	0.041	0.696	1.965	1.522	0.06	0.53
80 mg/kg/day	n	5	5	5	5	2	2	2	2
	Stat	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
BIA 2-093	Mean	8.712	21.195	0.410	12.225	11.370	22.682	0.50	12.75
Female	SD	1.082	1.442	0.031	0.512	0.113	0.038	0.01	0.00
160 mg/kg/day	n	4	4	4	4	2	2	2	2
	Stat	*	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.

n.a. = not applicable n.s. = not significant
* $p < 0.05$ when compared to vehicle-treated group of the same sex using Dunnett's test

Brain Weight and Dimensions: Brain weight, length, and width were not affected by the test article.

Histopathology: Adequate battery: No, nasal turbinates/ cavities were not examined. Surviving animals in the toxicokinetic group were subjected to whole body perfusion and an expanded battery of CNS and PNS tissues were collected and evaluated (see sponsor's list below); Peer Review: Yes; Signed and Dated Report: Yes.

Tissues collected for expanded nervous system histopathology assessment of animals in the TK group

Brain:	olfactory bulbs, cerebral cortex, hippocampus/dentate gyrus, basal ganglia, thalamus, hypothalamus, midbrain, (tectum, tegmentum, cerebral peduncles, and central gray matter), cerebellum, pons, and medulla oblongata
Spinal cord:	at cervical swellings C_3-C_7 and at lumbar swellings T_{13}-L_4
Trigeminal ganglia/nerves [a]	
Lumbar dorsal root ganglia at T_{13}-L_4 [b]	
Lumbar dorsal root fibers at T_{13}-L_4 [b]	
Lumbar ventral root fibers at T_{13}-L_4 [b]	
Cervical dorsal root ganglia at C_3-C_7 [b]	
Cervical dorsal root fibers at C_3-C_7 [b]	
Cervical ventral root fibers at C_3-C_7 [b]	
Sciatic nerves (mid-thigh region) (2) [c]	
Sciatic nerves (at sciatic notch) (2) [c]	
Sural nerves (2) [c]	
Tibial nerves (2) [c]	
Peroneal nerves (2) [c]	
Optic nerves [a]	
Eyes [a]	
Skeletal muscle (gastrocnemius)	
Other sites (if deemed necessary)	

[a] - Both tissues were processed.
[b] - Four to 6 tissues were collected at necropsy; two tissues were evaluated microscopically.
[c] - One tissue was processed for microscopic examination.
(2) - Two sections of the tissue were evaluated from the right hind leg. The tissues from the left hind leg were collected and preserved for possible future evaluation.

Histopathology findings from early decedents are discussed above in the “Mortality” section. There were limited test article-related findings in the animals that were euthanized at the end of the dosing period (sponsor’s table below). One HDM exhibited minimal nephropathy and another HDM exhibited minimal lung fibrosis. Hydrocephalus was observed in one HDF. Mild to moderate periarteritis (heart or ovary) was observed in 2 HDF. With the exception of minimal axonal degeneration in the spinal cord of 1/2 HDM, there were no test article-related findings in the animals euthanized at the end of the recovery period. Given that a single animal exhibited axonal degeneration, it is difficult to determine if this finding is drug-related. There were no test article-related findings in the expanded CNS and PNS histopathology assessment performed in the animals from the TK group.

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TABLE 40 (PND 329 PRIMARY NECROPSY)
PROJECT NO. (b) (4)-682002M A 10-MONTH ORAL STUDY OF BIA 2-093 IN JUVENILE DOGS PAGE 3
SPONSOR:BIAL-PORTELA & CA SUMMARY OF MICROSCOPIC FINDINGS

----- MALE -----

GROUP:	1	2	3	4
NUMBER OF ANIMALS IN DOSE GROUP	8	8	8	8
NUMBER OF ANIMALS EXAMINED	5	2	1	4
KIDNEYS				
TOTAL NUMBER EXAMINED	5	1	0	4
EXAMINED, UNREMARKABLE	5	1	0	3
-NEPHROPATHY	0	0	0	1
MINIMAL	NONE	NONE	NONE	1
LUNGS				
TOTAL NUMBER EXAMINED	5	0	0	4
EXAMINED, UNREMARKABLE	3	0	0	3
-FIBROSIS	0	0	0	1
MINIMAL	NONE	NONE	NONE	1
-INFILTRATE, MIXED INFLAMMATORY CELL	2	0	0	1
MINIMAL	2	NONE	NONE	1

----- FEMALE -----

GROUP:	1	2	3	4
BRAIN				
TOTAL NUMBER EXAMINED	5	0	0	4
EXAMINED, UNREMARKABLE	5	0	0	3
-HYDROCEPHALUS	0	0	0	1
PRESENT	NONE	NONE	NONE	1
HEART				
TOTAL NUMBER EXAMINED	5	0	0	4
EXAMINED, UNREMARKABLE	5	0	0	3
-PERIARTERITIS	0	0	0	1
MILD	NONE	NONE	NONE	1
OVARIES				
TOTAL NUMBER EXAMINED	5	0	0	4
EXAMINED, UNREMARKABLE	5	0	0	3
-PERIARTERITIS	0	0	0	1
MINIMAL	NONE	NONE	NONE	1

TABLE 41 (PND 385 RECOVERY NECROPSY)
PROJECT NO. (b) (4)-682002M A 10-MONTH ORAL STUDY OF BIA 2-093 IN JUVENILE DOGS PAGE 7
SPONSOR:BIAL-PORTELA & CA SUMMARY OF MICROSCOPIC FINDINGS

----- MALE -----

GROUP:	1	2	3	4
SPINAL CORD				
TOTAL NUMBER EXAMINED	3	0	0	2
EXAMINED, UNREMARKABLE	3	0	0	1
-DEGENERATION, AXONAL	0	0	0	1
MINIMAL	NONE	NONE	NONE	1

Toxicokinetics: Oral administration of BIA 2-093 resulted in systemic exposure to the parent compound, the main human metabolite (BIA 2-194), and oxcarbazepine (sponsor's tables, below). The majority of the systemic exposure was to BIA 2-194 (PND 21: Male= 72%-81%, Female 77%- 80%; PND 174: Male=78%-84%, Female= 80%-82%; PND 328: Male= 80%-85%, Female=83%-85%). There was an overall trend for males to exhibit higher exposure to BIA 2-093 and its metabolites. There was no individual TK information available for the HD animals that exhibited convulsions during the study.

Text Table 3. Summary of Toxicokinetic Parameters for BIA 2-093, BIA 2-194, and Oxcarbazepine in Male Juvenile Dogs

Dosage	$AUC_{0\text{-}t}$ (ng•h/mL)			Metabolite/Parent Ratio*			$AUC_{0\text{-}24\ h}$ (ng•h/mL)			C_{max} (ng/mL)			t_{max} (h)		
BIA 2-093 (mg/kg/day)	PND 21	PND 174	PND 328	PND 21	PND 174	PND 328	PND 21	PND 174	PND 328	PND 21	PND 174	PND 328	PND 21	PND 174	PND 328
Males	BIA 2-093 Results														
40	4,230	1,020	1,360†	NA	NA	NA	4,870	1,410	1,520†	2,940	810	904†	0.50	0.50	0.50†
80	9,020	1,600	2,350	NA	NA	NA	9,560	1,820	2,790	4,830	1,830	2,610	0.75	0.50	0.50
160	17,700	3,000	6,310	NA	NA	NA	20,100	3,660	6,550	7,400	2,410	6,750	1.3	0.50	0.50
	BIA 2-194 Results														
40	31,500	11,600	23,200†	7.4	14	18†	33,800	12,400	24,900†	9,010	4,870	7,910†	1.3	0.75	0.83†
80	97,000	37,000	56,400	12	21	29	97,000	38,500	58,600	22,600	17,900	22,400	1.9	0.50	0.75
160	283,000	57,200	162,000	16	26	27	283,000	63,400	166,000	40,100	18,700	50,800	2.3	2.1	1.6
	Oxcarbazepine Results														
40	6,740	1,900	3,940†	1.7	2.2	3.3†	8,140	2,050	4,410†	1,520	669	1,200†	2.6	1.0	1.5†
80	16,200	5,070	8,250	1.9	2.9	4.3	18,400	5,420	8,820	3,140	1,860	2,490	3.0	1.5	1.3
160	26,800	8,610	20,700	1.5	3.8	3.4	42,100	9,800	22,000	4,150	2,450	5,190	4.5	2.4	1.9

N =4, except where †N = 3
* = Ratio of Metabolite $AUC_{0\text{-}t}$/BIA 2-093 $AUC_{0\text{-}t}$
NA = Not Applicable

Text Table 4. Summary of Toxicokinetic Parameters for BIA 2-093, BIA 2-194, and Oxcarbazepine in Female Juvenile Dogs

Dosage	$AUC_{0\text{-}t}$ (ng•h/mL)			Metabolite/Parent Ratio*			$AUC_{0\text{-}24\ h}$ (ng•h/mL)			C_{max} (ng/mL)			t_{max} (h)		
BIA 2-093 (mg/kg/day)	PND 21	PND 174	PND 328	PND 21	PND 174	PND 328	PND 21	PND 174	PND 328	PND 21	PND 174	PND 328	PND 21	PND 174	PND 328
Females	BIA 2-093 Results														
40	3,860	1,050†	974	NA	NA	NA	4,300	1,370†	1,190	3,590	839	1,210	0.75	0.50	0.50
80	6,570	2,050	1,410†	NA	NA	NA	7,290	2,440	1,580†	4,800	3,040	2,360†	0.50	0.50	0.50†
160	13,900	3,250	3,480	NA	NA	NA	15,200	3,710	3,880	7,170	2,660	2,090	0.75	0.50	0.75
	BIA 2-194 Results														
40	37,400	13,400	19,100	11	13†	20	38,600	14,300	20,400	14,100	7,480	10,800	1.5	0.75	0.75
80	69,400	28,600	40,500†	11	16	29†	72,600	29,700	42,800†	18,300	17,200	23,600†	1.9	0.50	0.50†
160	191,000	64,200	76,000	12	23	23	191,000	70,000	77,700	32,400	26,100	23,200	2.3	1.0	1.4
	Oxcarbazepine Results														
40	6,590	1,960	2,640	1.9	2.0†	2.8	7,110	2,100	3,040	1,820	934	1,120	2.6	1.0	1.0
80	11,800	3,850	5,390†	1.9	2.2	3.8†	13,100	3,980	5,910†	2,650	1,790	2,420†	3.0	1.0	0.83†
160	26,900	9,170	9,570	2.0	3.2	2.8	31,500	10,200	10,800	4,060	3,100	2,930	3.8	1.3	1.9

N =4, except where †N = 3
* = Ratio of Metabolite $AUC_{0\text{-}t}$/BIA 2-093 $AUC_{0\text{-}t}$
NA = Not Applicable

A comparison of exposure to eslicarbazepine acetate (SEP-002093), eslicarbazepine, and oxcarbazepine in juvenile and adult dogs dosed daily with BIA 2-093 demonstrates that exposure at the LD (40 mg/kg) was similar in juvenile males and adult males. Exposure to BIA 2-093 and its metabolites was higher in LD and MD adult females and MD adult males relative to juveniles. The reviewer does not agree with the sponsor's characterization of 40 mg/kg/d and 80 mg/kg/d as the NOAEL and LOAEL, respectively, due to deaths at all dose levels.

Table 6: Comparison of Systemic Exposures at the NOAEL and LOAEL in Juvenile and Adult Dogs in Long-Term Toxicity Studies

Analyte	Systemic Exposure[a]							
	Juvenile Dogs (PND 328)[b]				Adult Dogs (Week 52)[c]			
	C_{max} (ng/mL)		$AUC_{0.5-24}$ (ng·h/mL)		C_{max} (ng/mL)		$AUC_{0.5-24}$ (ng·h/mL)	
	M	F	M	F	M	F	M	F
NOAEL (40 mg/kg/day)								
SEP-0002093	904[d]	1210	1520[d]	1190	1008	906	1662	1397
Eslicarbazepine[e]	7910[d]	10800	24900[d]	20400	9229	13650	29987	33121
Oxcarbazepine	1200[d]	1120	4410[d]	3040	1250	1542	4776	5104
LOAEL (80 mg/kg/day)								
SEP-0002093	2610	2360	2790	1580[d]	2442	1986	4518	2896
Eslicarbazepine[e]	22400	23600[d]	58600	42800[d]	26079	34229	73478	77072
Oxcarbazepine	2490	2420[d]	8820	5910[d]	2546	3785	8919	11396

[a] M = Male; F = Female
[b] Data from Document 093-866
[c] Data from Document 093-819
[d] N = 3
[e] A chiral assay was used to evaluate exposure to eslicarbazepine and (R)-licarbazepine in juvenile dogs. However, an achiral assay was used to evaluate licarbazepine exposure in the study in adult dogs. As the ratio of eslicarbazepine to (R)-licarbazepine in dog following administration of SEP-0002093 is at least 88 to 1 (Document 093-858), nearly all licarbazepine exposure is to eslicarbazepine.

11 Integrated Summary and Safety Evaluation

This review represents the evaluation of the nonclinical information provided in the sponsor's resubmission of NDA 22-416 (dated 9/4/2102 and 2/11/2013), which was submitted in response to the Complete Response (CR) Letter dated 4/30/13. A full review of the information provided in the initial NDA submission (received 3/30/09) can be found in the nonclinical review filed in DARRTS on 4/14/2010. In the CR letter, the Division requested that the sponsor either demonstrate that the completed genetic toxicology studies were adequate or conduct an *in vitro* chromosomal aberration assay in mammalian cells or an *in vitro* mouse lymphoma tk assay in the presence and absence of an appropriate metabolic activation system. In the resubmission of NDA 22-416, the sponsor provided a nonclinical package which included primary pharmacology studies, CNS safety pharmacology studies, genetic toxicology studies, juvenile toxicology studies, and environmental toxicology studies; the environmental toxicology studies were not reviewed.

In the primary pharmacology studies provided in the initial submission of the NDA, the sponsor demonstrated that the major human metabolite of eslicarbazepine acetate (BIA 2-093), eslicarbazepine (BIA 2-194, S-licarbazepine), and the two minor human metabolites, R-licarbazepine (BIA 2-195) and oxcarbazepine (OXC), are antagonists of voltage-gated sodium channels. The primary pharmacology studies provided in the resubmission support the findings of the previously reviewed primary pharmacology studies. Specifically, *in vitro* studies conducted with CHO cells expressing voltage-gated sodium channels demonstrate that rat $Na_V1.2$ and human $Na_V1.3$ are inhibited by BIA 2-194, BIA 2-195, and OXC. OXC exhibited the greatest potency (IC_{50}: $Na_V1.2$= 81 µM; $Na_V1.3$= 100 µM) of the three compounds at the sodium-gated channels, with IC_{50} values that were 3-4 fold lower than BIA 2-194 and BIA 2-195 at the rat $Na_V1.2$ and 1.5-2.6-fold lower than these BIA 2-093 metabolites at the human $Na_V1.3$. BIA 2-194 also exhibited activity at the $Ca_V3.2$ T-type channel, with an IC_{50} of 380 nM; the potency of BIA 2-195 at the voltage-gated calcium channel was 12-fold lower than BIA 2-194. Primary pharmacology studies provided in the initial NDA submission demonstrated that OXC is also an inhibitor of calcium channels (Study # 093-417 & 093-427). OXC, CBZ, BIA 2-093, and BIA 2-194 exhibited similar activity in animal models of seizure such as fully kindled mice, the maximal electroconvulsive shock test, and the 6Hz psychomotor test. Overall, the pharmacologic activity exhibited by the metabolites of eslicarbazepine acetate is similar to previously approved structural analogs such as CBZ and OXC. Therefore, it is recommended that eslicarbazepine acetate be labeled using the same established pharmacologic class (EPC) as the CBZ structural derivative, OXC. The current EPC and FDA text phrase for OXC are "Anti-epileptic agent" and "antiepileptic drug", respectively. Proposed labeling for the "Highlights of Prescribing Information" and mechanism of action sections of the label are provided in the previous nonclinical review and do contain the current OXC EPC in the description of BIA 2-093's pharmacologic activity (Toscano CD, 4/14/2010).

Although the sponsor provided in the initial NDA submission, the results of a CNS safety pharmacology (Irwin study) assessment of BIA 2-093 in mice, the results of an additional Irwin assessment of BIA 2-093, BIA 2-194, OXC, and CBZ were provided in the resubmission. The dose response for specific clinical signs differed between test articles in the Irwin test, but the clinical signs and findings in the Irwin test were similar

for BIA 2-093, BIA 2-194, OXC, and CBZ. A dose-dependent decrease in time to drop-off was observed in mice dosed with any of the four compounds before assessment in the Rotarod assay. The findings in these recently submitted CNS safety pharmacology studies are consistent with the findings of the CNS safety pharmacology study described in the initial NDA submission.

As detailed in the nonclinical review of the initial NDA submission, rat is not an appropriate animal model for assessing the toxicity of BIA 2-093, mainly because of the propensity of this species to form OXC as the major metabolite. The main human metabolite of BIA 2-093 is BIA 2-194 (eslicarbazepine). In the initial NDA submission, the sponsor provided study reports for *in vitro* genetic toxicology studies conducted with rat hepatic S9 fraction as the metabolic activating system; an *in vivo* assessment was performed in mouse. The mutagenicity of BIA 2-194 was directly tested in an *in vitro* bacterial reverse mutagenicity assay and the results of this assay were reported in the initial NDA submission. The overall approach employed by the sponsor in the initial submission did not adequately assess the potential for BIA 2-194, the main human metabolite of BIA 2-093, to cause chromosomal aberrations in an *in vitro* assay. Given the inadequate testing for chromosomal aberration in an *in vitro* assay, the Division requested in the CR letter that the sponsor either demonstrate the adequacy of the previously conducted *in vitro* chromosomal aberration studies or perform an additional study conducted in the presence of an appropriate metabolic activation system. In the resubmission, the sponsor provided study reports for *in vitro* bacterial reverse mutation and chromosomal aberration assays in which human hepatic S9 fraction was used as the metabolic activation system. These studies were adequately conducted and demonstrated that BIA 2-194, when incubated in the presence or absence of human hepatic S9 fraction, was not mutagenic in bacteria and did not cause chromosomal aberration in human peripheral blood lymphocytes. The genetic toxicology study reports provided in the resubmission of the NDA fulfill the Division's nonclinical request made in the CR letter.

The sponsor has requested approval to market eslicarbazepine acetate as an "adjunctive therapy in the treatment of partial-onset seizures in patients 18 years and older." Therefore, at the current time, the sponsor is not seeking approval for the use of eslicarbazepine in a pediatric population. However, the sponsor has submitted two studies, one dose-ranging and one pivotal, in which juvenile dogs were dosed daily with eslicarbazepine acetate for 1 or 10 months, respectively, beginning on postnatal day (PND) 21. Based on the fact that the sponsor is not currently seeking approval for a pediatric indication, juvenile toxicology studies are not required for approval of NDA 22-416. However, these studies were reviewed in order to determine if they support the proposed labeling for Section 8.4.

The Division commented on and "generally concurred" with the design of the juvenile toxicology studies (IND 67466: Information Request 8/14/2008; Meeting Minutes 1/12/2009). Specifically, the Division stated that a dosing duration of 10 months with a recovery period of 2 months was considered appropriate to adequately address the impact of the test article on sexual maturity in beagle dogs. The Division did not explicitly comment on the dose levels of BIA 2-093 to be used in the pivotal juvenile toxicology study. In the dose range-finding study conducted in juvenile beagle dogs (Study #093-800), animals were dosed with 25, 100, or 200 mg/kg/day BIA 2-093, by

oral gavage for 28 days, beginning on PND 21. The main test article-related findings were clinical signs such as tremors, convulsions, impaired muscle coordination, and hypoactivity. The NOAEL in this study, 25 mg/kg/day, was not chosen as one of the dose levels in the pivotal juvenile toxicology study (Study #093-866). Rather, the dose levels chosen for the pivotal study were 40, 80, and 160 mg/kg BIA 2-093; the sponsor did not provide justification for not including the NOAEL from the dose range-finding study in the pivotal study. Deaths (found dead or euthanized *in extremis*) occurred at all doses of BIA 2-093 (0 Controls, 2 LD, 2 MD, 5 HD) in the pivotal juvenile toxicology study. With the exception of the LD animal (5583-07) that exhibited continuous convulsions, the deaths that occurred in the pivotal study are considered by this reviewer to be test article-related; therefore a NOAEL could not be determined in this study. Of the early decedents in the pivotal study, 1/2 LD, 2/2 MD, and 5/5 HD exhibited lymphoid depletion in tissues such as lymph nodes, thymus, and Peyer's patches. In addition, 1/2 LD, 2/2 MD, and 3/4 HD animals exhibited depletion of the bone marrow; femoral bone marrow was not examined in one of the HD early decedents (animal #5574-03). Lymphoid and marrow depletion were not observed in animals that survived to the end of the dosing period, in concurrent controls, or in adult beagle dogs dosed at the same dose levels for up to 12 months (Study #'s 093-815, 093-816, 093-817, 093-818, 093-819). The Pathology report provided in the pivotal juvenile toxicology study states that "Systemic stress, as evidenced by the weight losses noted above, could cause lymphoid depletion and bone marrow hypocellularity." Although decreased BW or BW gain and depletion of lymphocytes in the thymus and spleen are possible outcomes of the stress response [1], other sequelae associated with stress such as increased adrenal gland weight, increased neutrophils, and decreased eosinophils were not observed in the early decedents. Given the dose-dependent occurrence of lymphoid and marrow depletion and the lack of conclusive evidence that these findings were stress-related, the deaths and associated findings of lymphoid and bone marrow depletion are considered to be test article-related and not related solely to stress. Therefore, it is recommended that these findings be discussed in the eslicarbazepine acetate labeling. Furthermore, the sponsor should be informed of the need to determine a NOAEL in juvenile animals dosed with eslicarbazepine acetate before conducting clinical trials in a pediatric population. This decision is based on the fact that there were deaths at every dose level in the pivotal study.

Although convulsions were described in juvenile animals but not in adult animals dosed with 160 mg/kg/d for up to 12 months, the adult animals did exhibit, what was described by the sponsor to be, "striking, seizure-like clinical signs." While it may seem that juvenile animals have an increased sensitivity to eslicarbazepine-induced convulsions; it is also possible that a difference in the terminology used in the two labs that conducted the studie (b) (4) may account for the perceived finding of juvenile susceptibility to convulsions. Complicating the assessment of the potential differences between adults and juveniles with regard to convulsions, TK information was not available for most of the individuals that exhibited convulsions in the pivotal study conducted in juvenile animals. The LD early decedent that exhibited continuous convulsions had, on Day PND 174, a C_{max} of BIA 2-194 and OXC that were almost twice as high as the other LD animals in this study, thereby suggesting that the

convulsions in this animal may have been because of a greater exposure to the BIA 2-093 metabolites in this animal. The reason for the higher plasma levels of BIA 2-093 metabolites in this animal is not known; there is no mention of dosing error for this animal in the deviations section of the study report. Given the lack of individual TK information for the HD animals that exhibited convulsions in the pivotal juvenile study, it was not possible to determine if higher plasma exposures to eslicarbazepine acetate or its metabolites were achieved in these animals. In the opinion of this reviewer, the neurobehavioral signs observed in juvenile and adult animals dosed with 160 mg/kg BIA 2-093 do not differ to the extent that would indicate a unique susceptibility of juvenile animals to eslicarbazepine acetate-induced neurotoxicity.

Since the sponsor was not able to determine a NOAEL for daily exposure to BIA 2-093 in juvenile animals, it is recommended that if the sponsor does, at some point, seek approval for dosing in a pediatric population, additional assessment of the toxicity of BIA 2-093 in juvenile animals should be required. Future studies of the toxicity of BIA 2-093 in juvenile animals should identify a NOAEL in order to support the safety of BIA 2-093 in pediatric populations. It should be recognized that the sponsor has identified a NOAEL in the 28-day dose range-finding study (25 mg/kg). Although the duration of dosing for the dose range-finding study is inadequate to determine the safety of dosing in pediatric populations, the sponsor should use the information gathered from the dose-range finding study to determine an adequate dose range for future juvenile toxicology studies. Overall, the inability to identify a NOAEL in juvenile dogs dosed with BIA 2-093 does not preclude the approval of eslicarbazepine acetate for the proposed indication. However, the sponsor's proposed labeling for eslicarbazepine acetate should be amended to mention the occurrence of deaths, lymphoid depletion, and bone marrow depletion at all dose levels in the pivotal study.

In summary, the sponsor has fulfilled the nonclinical request made in the Complete Response letter (4/30/10) by providing adequate assessments of the genetic toxicology of eslicarbazepine in the presence and absence of human hepatic S9 fraction. As these were the only outstanding nonclinical studies, the resubmission along with the initial submission represents a complete assessment of the toxicology of eslicarbazepine acetate. In considering the totality of the nonclinical studies provided in the initial submission and resubmission, the nonclinical information on eslicarbazepine acetate supports the approval of NDA 22416. The sponsor should be informed of the need to perform additional assessment of the toxicity of eslicarbazepine in juvenile animals in case the sponsor seeks approval of a pediatric indication.

12 References

1. Everds NE, Snyder PW, Bailey KL, Bolon B, Creasy DM, Foley GL, Rosol TJ, Sellers T. Interpreting stress responses during routine toxicity studies: a review of the biology, impact, and assessment. Toxicol Pathol. 2013;41(4):560-614.

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/s/

CHRISTOPHER D TOSCANO
09/06/2013

LOIS M FREED
09/06/2013

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 22-416 **Applicant: Sunovion Pharmaceuticals, Inc.** **Stamp Date: 2/11/2013**

Drug Name: Stedesa **NDA/BLA Type: 505(b)(1)**

On **initial** overview of the NDA 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?	N/A	N/A	See comment below regarding this Class 2 Resubmission.
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	N/A	N/A	----------
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	N/A	N/A	----------
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)?	N/A	N/A	----------
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).	N/A	N/A	----------
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?	N/A	N/A	----------
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?	N/A	N/A	----------
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	N/A	N/A	----------

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

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?	N/A	N/A	----------
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	N/A	N/A	----------
11	Has the applicant addressed any abuse potential issues in the submission?	N/A	N/A	----------
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?	N/A	N/A	----------

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

Comment: There are no new nonclinical study reports included in the 2/11/2013 submission. However, the Sponsor's previous submission (9/4/12) contained new nonclinical study reports; the 9/4/12 submission was previously determined to be fileable (see Nonclinical Filing Review in DARRTS 9/24/12).

Christopher D. Toscano, Ph.D., DABT 2/22/12
Reviewing Pharmacologist Date

Lois M. Freed, Ph.D.
Team Leader/Supervisor Date

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

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/s/

CHRISTOPHER D TOSCANO
02/22/2013

LOIS M FREED
02/22/2013

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 22-416 **Applicant: Sunovion Pharmaceuticals, Inc.** **Stamp Date: 9/4/12**

Drug Name: Eslicarbazepine Acetate **NDA Type: 505(b)(1)**

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		Format and organization is adequate. The pivotal toxicology study performed in juvenile dog contains a signed and dated pathology report.
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		Indexing and pagination are adequate.
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		Line listings are provided for each study and are legible.
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		Due to the metabolic profile in rat being markedly different from human, it was requested in the CR letter that the Sponsor conduct either an *in vitro* chromosomal aberration assay in mammalian cells or an *in vitro* mouse lymphoma tk assay with an appropriate metabolic activation system. In the resubmission, the Sponsor has provided study reports for 3 genotoxicity studies (2 Ames studies and 1 chromosomal aberration study) in which the metabolic activation system is comprised of human liver S9.
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?	X		The drug product is formulated as a tablet. Animals were dosed by oral gavage.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route?	X		Route of administration in the pivotal juvenile toxicology study was oral gavage.
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		All pivotal studies were GLP-compliant.
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		The genetic toxicology study (*in vitro* chromosomal aberration) requested in the CR letter is included in the resubmission.

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		The proposed nonclinical labeling expresses human dose multiples based on body surface area. However, the "*Indications and Usage*" section of "*Highlights of Prescribing Information*" is not in the format recommended in "*Guidance for Industry and Review Staff: Labeling for Human Prescription Drug and Biological Products- Determining Established Pharmacologic Class for Use in the Highlights of Prescribing Information, October 2009)*".
10	Have any impurity – etc. issues been addressed?	X		

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

Christopher D. Toscano, Ph.D, DABT — 9/20/12

Reviewing Pharmacologist — Date

Lois M. Freed, Ph.D.

Team Leader/Supervisor — Date

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/s/

CHRISTOPHER D TOSCANO
09/24/2012

LOIS M FREED
09/24/2012

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 22-416
Submission date: March 30, 2009
Drug: Stedesa (eslicarbazepine acetate)
Applicant: Sepracor
Indication: Adjunctive treatment of partial-onset seizures in adults with epilepsy

Reviewing Division: Division of Neurology Products

Introductory Comments:
The primary pharmacology/toxicology reviewer and supervisor agree that the nonclinical information submitted is adequate to support approval of eslicarbazepine acetate for the indication noted above. However, the pharmacology/toxicology reviewer noted a deficiency in the genotoxicity data that he concluded should be addressed by the applicant. The supervisor considered this issue in a secondary review and agreed that the deficiency should be addressed, although the supervisor differed slightly in how it could be addressed.

Metabolism:
Eslicarbazepine acetate is metabolized primarily (95%) to eslicarbazepine in humans. Eslicarbazepine is also the primary metabolite in mice (74%) and dogs (88%), but is not the primary metabolite in rats (13%).

Reproductive and developmental toxicity:
A full battery of reproductive and developmental toxicity studies was conducted in mice and an embryo-fetal study was conducted in rabbits. Eslicarbazepine acetate was teratogenic in mice and rabbits. A full battery of studies was also conducted in rats, although these studies would not have fully addressed the effects of eslicarbazepine because of the metabolism issues discussed above.

Carcinogenicity:
A 2-year carcinogenicity of eslicarbazepine acetate was conducted in CD-1 mice. A carcinogenicity study was not conducted in rats with agreement from the division because of the difference in metabolism between rats and humans. The executive carcinogenicity assessment committee found that the mouse study was an adequate study and that the incidences of hepatic adenomas and hepatocellular carcinomas in males and females were increased and drug-related.

Genetic toxicity:
Adequate bacterial mutagenicity assays were conducted with eslicarbazepine acetate and its primary human metabolite, eslicarbazepine. An adequate in vivo mouse micronucleus assay was conducted with eslicarbazepine acetate, which would also have tested eslicarbazepine since this metabolite is produced at

significant levels in the mouse. In vitro mammalian cell genotoxicity assays were conducted with eslicarbazepine acetate using rat liver S9 fractions as the metabolic activation system. The applicant did not confirm that eslicarbazepine was formed in these assays. Therefore, the pharmacology/toxicology reviewer concluded that the genotoxic potential of eslicarbazepine acetate (and its primary human metabolite, eslicarbazepine) was not adequately characterized. The pharmacology/toxicology reviewer recommended that the applicant complete, as a post marketing requirement (PMR), both the in vitro mouse lymphoma mutation assay and the in vitro cytogenetic assay in Chinese Hamster Ovary (CHO) cells with the main human metabolite, eslicarbazepine.

The pharmacology/toxicology supervisor has considered the genetic toxicity of eslicarbazepine in her secondary review. She agrees that it is not clear whether the in vitro mammalian cell assays have adequately evaluated the genotoxic potential of eslicarbazepine acetate because of the possible lack of eslicarbazepine in these assays. She recommended that the genetic toxicity of eslicarbazepine be evaluated in either an in vitro mouse lymphoma tk assay or an in vitro chromosomal aberration assay. Alternatively, the supervisor notes that the applicant could also address this issue by providing information showing that eslicarbazepine was present at adequate levels in the completed in vitro mammalian cell assays. The supervisor noted that completing this assessment is important, in part, because of the positive tumor signal observed in the mouse study and the absence of a rat carcinogenicity study. It was also noted that another drug with similar tumor findings was positive for genotoxicity only in the in vitro mammalian cell assays.

Conclusions:
I have discussed this NDA with the division pharm/tox supervisor. I agree that the nonclinical information is adequate to support approval of eslicarbazepine acetate for the proposed indication. I note that eslicarbazepine acetate has been adequately assessed in 2 of the three types of genotoxicity studies recommended by ICH and an adequate carcinogenicity study has been completed. The recommended genotoxicity evaluation will provide a relatively small additional amount of information but may help in understanding the potential carcinogenic mode of action and so I agree that the applicant can be asked to address the shortcomings of the already conducted assays. As noted above, characterization of the genotoxicity of eslicarbazepine in an in vitro mammalian cell assay need not be a pharm/tox approval issue. This information would be included in labeling but would not likely lead to a different recommendation on approvability.

Labeling will be addressed at a later time.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22416	ORIG-1	SEPRACOR INC	SEP-0002093 ESLICARBAZEPINE ACETATE

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/s/

PAUL C BROWN
04/29/2010

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health
Service
Food and Drug
Administration

Division of Neurology Products (HFD-120)
Center for Drug Evaluation and Research

Date: April 28, 2010

From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 22-416 (Stedesa, eslicarbazepine acetate, SEP-0002093, BIA 2- 093): N0000, 30 March 2009

NDA 22-416 was submitted by the sponsor (Sepracor, Inc.) for the use of eslicarbazepine acetate as adjunctive therapy for treatment of partial-onset seizures in adults with epilepsy. To support the development and marketing of eslicarbazepine acetate for this indication, the sponsor has submitted a full battery of nonclinical studies (under IND 67466), with the exception of a 2-year carcinogenicity study in rats. These nonclinical data have been reviewed in detail by Dr. Toscano (Pharmacology/Toxicology NDA Review and Evaluation, Christopher D. Toscano, Ph.D., 4/14/2010).

Based on his review, Dr. Toscano has concluded that the nonclinical data support approval, but that the sponsor should "…be required to conduct an *in vitro* mammalian mutagenicity assay and an *in vitro* chromosomal aberration assay on the main human metabolite, BIA 2-194 (eslicarbazepine)" as a post-marketing requirement.

- **Summary of nonclinical findings**

The following is based primarily on information provided in Dr. Toscano's review.

Pharmacology: Eslicarbazepine acetate is a pro-drug, which is rapidly hydrolyzed *in vivo* to the active metabolite, eslicarbazepine. Based on mechanistic studies, eslicarbazepine is characterized as a blocker of voltage-gated calcium and sodium channels, and has demonstrated anticonvulsant activity in various animal seizure models.

Eslicarbazepine is the (S)-enantiomer of the active (mono-hydroxy) metabolite of oxcarbazepine. Oxcarbazepine [Trileptal] is approved as adjunctive and monotherapy for partial seizures in adults and as adjunctive therapy in children, 2-16 yrs of age.

PK/ADME/TK: in mouse, dog, rabbit, and human, eslicarbazepine acetate (the prodrug) is metabolized *in vivo* primarily to the active metabolite, eslicarbazepine. In contrast, the rat metabolizes the prodrug primarily to oxcarbazepine. These differences are illustrated in the following sponsor's Table 19:

Table 19: Metabolite Profile Following Oral Administration of SEP-0002093 to Various Species

	% of Circulating Species that Correspond to Parent and Metabolite Following Oral Administration of SEP-0002093*			
	SEP-0002093 [BIA 2-093]	Oxcarbazepine	Eslicarbazepine [BIA 2-194]	(R)-Licarbazepine [BIA 2-195]
Human	ND	1%	95%	4%
Dog	8%	4%	88%	ND
Rabbit	ND	7%	93%	<1%
Rat	ND	86%	13%	1%
Mouse	ND	25%	74%	<1%

*Data are taken from the following studies: BIA-2093-105 (human); Sepracor Document No. 093-858 (dog); Sepracor Document No. 093-517 (rabbit); Sepracor Document No. 093-813 (rat); Sepracor Document No. 093-847 (mouse); ND = not detected

Following multiple oral doses of 800 and 1200 mg/day in humans (Study BIA-2093-301), plasma levels (C_{max}, AUC) of eslicarbazepine were ≈60-70 times higher than those of oxcarbazepine. In comparison, the plasma exposure (C_{max}, AUC) ratios of eslicarbazepine-to-oxcarbazepine in mouse, rabbit, and rat were 4-3, 20-13, and ≤0.2, respectively. For dog, eslicarbazepine was not measured directly since only an achiral assay was used. However, the sponsor provided estimates of eslicarbazepine AUC (but not C_{max}), based on the finding that (R)-licarbazepine was low-to-not detectable in plasma collected during a single-dose safety pharmacology study (#093-858). Using those estimates, the AUC ratio in dogs was 6-7. The data are provided in the following table:

SPECIES	SEX	STUDY	SAMPLING TIME	DOSE (mg/kg)	ESLICARBAZEPINE		OXCARBAZEPINE	
					C_{max} (ng/mL)	AUC (ng•hr/mL)	C_{max} (ng/mL)	AUC (ng•hr/mL)
mouse	M/F	093-847	GD15	650	51300	268000	13000	91200
rabbit	M/F	093-517	GD18	320	69000	230000	3490	17700
rat	M	096-813	Week 15	300	2320	21300	11000	138000
	F				2520	27300	13600	248000
dog	M	093-819	Week 52	160	--	67400	--	9850
	F				--	112400	--	17500
human	M/F	2093-301	--	800 mg	15462	205359	232	2921
				1200 mg	22957	336147	385	5076

Due to the marked difference in *in vivo* metabolism in the rat compared to human, the sponsor conducted repeat-dose (up to 13 weeks) oral toxicity studies and a full battery of reproductive and developmental toxicology studies in the mouse. Waiver of the 2-year carcinogenicity study in rat (IND 67466: Agency communication, 7/20/2007) was based on this difference, although not all of these data were available at the time. (TK analyses in most of the pivotal nonclinical studies were conducted using an achiral assay.)

Repeat-dose oral toxicity: Pivotal oral toxicity studies of up to 3 and 12 months in duration were conducted in CD-1 mouse and Beagle dog, respectively. Chronic toxicity data for the mouse were obtained in the 2-year carcinogenicity study. The most notable toxicities were CNS signs (e.g., unsteady/abnormal gait) and centrilobular hypertrophy in the mouse and "seizure-like" behavior in the dog; also, QT_c shortening was a consistent finding in dog.

[Oral toxicity studies of up to 26-weeks duration were conducted in rat.]

Reproductive and Developmental Toxicology: a full battery of reproductive and developmental toxicology studies was conducted in CD-1 mouse (fertility and early embryonic development, embryo-fetal development, pre/post-natal development) and New Zealand White rabbit (embryo-fetal development). Eslicarbazepine acetate was teratogenic in mouse (major abnormalities, including cleft palate, were observed at all doses tested) and rabbit (major skeletal abnormalities at the highest dose tested; irregular ridging of the palate in treated but not control fetuses).

[A full battery of reproductive and developmental toxicology studies was also conducted in Sprague-Dawley rat.]

Carcinogenicity: The carcinogenic potential of eslicarbazepine acetate was tested only in CD-1 mouse. Assessment of carcinogenic potential in a second species (rat) was waived due to the differences in *in vivo* metabolism between rat and human, described above. At the highest dose tested chronically in rat, plasma eslicarbazepine levels were ≈10-15% of that in humans at the sponsor's proposed maximum (1200 mg) and maintenance (800 mg) clinical doses. In contrast, plasma oxcarbazepine levels in rat were ≈50-80 and ≈30-50 times higher than that in humans at 800 and 1200 mg, respectively. Therefore, any tumor findings in a 2-year study in rat would not have been interpretable in terms of the relevance to humans.

In the 2-year mouse study, the primary tumor finding was an increase in the incidence of hepatic adenomas and hepatocellular adenomas in males (mid and high doses) and females (high dose). The sponsor attributed this finding to a phenobarbital-like mechanism, i.e., enzyme induction. However, as Dr. Toscano

discusses in his review, the sponsor did not provide sufficient data to confirm this mechanism for eslicarbazepine.

Genetic Toxicology:

A full battery of genetic toxicology studies was conducted. Eslicarbazepine acetate, eslicarbazepine, R-licarbazepine, and oxcarbazepine were tested in separate *in vitro* Ames assays, in the presence and absence of metabolic activation (rat liver S9); all assays were negative. Eslicarbazepine acetate was tested in the *in vitro* mouse lymphoma tk and chromosomal aberration (CHO, HPBL) assays (with and without rat liver S9), and the *in vivo* micronucleus assay in mouse. The *in vivo* assay was negative, but the *in vitro* mammalian cell assays were equivocal or positive. Eslicarbazepine was not tested directly in an *in vitro* mammalian cell assay.

The one deficiency noted by Dr. Toscano is that the sponsor did not demonstrate the relevance of the *in vitro* mammalian cell assays. That is, all of the assays used rat liver S9. The sponsor was asked during clinical development to justify the adequacy of these assays (IND 67466: Agency letter, 1/26/2007; email communication, 4/20/2007), but has not done so. Considering the positive tumor findings in the 2-year mouse carcinogenicity study and the lack of an assessment of carcinogenic potential in a second species, it is not unreasonable to ask the sponsor to address this issue. While an adequate assessment of genotoxic potential is certainly not critical at this time in order to predict carcinogenic potential, it is important in terms of providing a better understanding of the mechanism underlying the liver tumors observed in the mouse.

[In another recent application, data from the *in vitro* mammalian cell assays were critical in identifying genotoxic potential for a drug producing a pattern of tumors similar to those observed with eslicarbazepine acetate. While the *in vitro* Ames and *in vivo* micronucleus assays were negative, the *in vitro* chromosomal aberration and mouse lymphoma tk assays were clearly and consistently positive.]

While Dr. Toscano recommends requiring the sponsor to conduct both an *in vitro* mouse lymphoma tk assay and an *in vitro* chromosomal aberration assay in mammalian cells, it is my opinion that assessing eslicarbazepine directly in either of these assays would be sufficient. Alternatively, the sponsor may attempt to document that the genotoxic potential of eslicarbazepine has been adequately characterized in the completed studies. While I do not believe this is an approvability issues (neither does Dr. Toscano), I would recommend that the sponsor not wait until all clinical issues have been resolved to address this nonclinical deficiency.

- **Conclusions and Recommendations**

I agree with Dr. Toscano that the sponsor's nonclinical data support approval of the NDA. While not an approvability issue, the sponsor has not, as Dr. Toscano notes, adequately addressed the Division's concerns regarding assessment of the genotoxic potential of eslicarbazepine. The sponsor should be asked in the action letter to address this issue.

Recommended wording to the sponsor:

You have not fully addressed our previous request (Agency letter dated 1/26/2007) for documentation of the adequacy of the in vitro assays for assessing the genotoxic potential of eslicarbazepine, the major circulating metabolite in humans. You have conducted an *in vitro* Ames assay testing eslicarbazepine directly and an *in vivo* micronucleus assay in the mouse (a species in which eslicarbazepine is a major circulating metabolite). However, you have not demonstrated that eslicarbazepine was adequately tested in the *in vitro* chromosomal aberration assays in mammalian cells or the *in vitro* mouse lymphoma tk assay since, in these assays, the metabolic activation system (liver S9) was from rat. The metabolic profile in rat is sufficiently different from human that the need for carcinogenicity assessment in this species was waived.

Due to the increase in hepatic tumors observed in the 2-year mouse carcinogenicity study, and considering the lack of an assessment of carcinogenic potential in a second species, a full characterization of the genotoxic potential of eslicarbazepine is important. We ask that you address this either by demonstrating that the completed assays are adequate or by conducting either an *in vitro* chromosomal aberration assay in mammalian cells or an *in vitro* mouse lymphoma tk assay (with colony sizing), testing eslicarbazepine directly with and without an appropriate metabolic activation system.

- **Recommended labeling**: the nonclinical sections of labeling are not being included in the Agency's action letter; therefore, labeling recommendations are not being made at this time.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22416	ORIG-1	SEPRACOR INC	SEP-0002093 ESLICARBAZEPINE ACETATE

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

/s/

LOIS M FREED
04/28/2010

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application Number:	22416
Submission Number/code:	000
CDER Stamp Date:	3/30/09
PDUFA Date:	4/30/10
Product:	Eslicarbazepine acetate (BIA 2-093)
Proposed Name:	STEDESA®
Indication:	Adjunctive treatment of partial-onset seizures in adults with epilepsy
Applicant:	Sepracor, Inc.
Review Division:	Neurology Products
Reviewer:	Christopher D. Toscano, PhD, DABT
Supervisor/Team Leader:	Lois M. Freed, PhD
Division Director:	Russell G. Katz, MD
Project Manager:	Dorothy Demczar, PharmD

Disclaimer: All figures and tables provided in this review prepared by the Sponsor are identified as such.

TABLE OF CONTENTS

1. Executive Summary

1.1. Recommendations

1.1.1. Approvability:

With the exception of the deficiency discussed in Section 1.1.2 of this review, NDA 22416 contains an adequate, non-clinical assessment of the pharmacology and toxicology of eslicarbazepine acetate (BIA 2-093). Although eslicarbazepine acetate has been demonstrated by the Sponsor to be both a teratogen (cleft and irregular ridging of the palate, exencephaly, increased number of vertebrae) and a carcinogen (hepatocellular carcinoma) in nonclinical studies, the overall nonclinical toxicity profile of eslicarbazepine acetate is consistent with other FDA-approved antiepileptics. With this in mind, it is the opinion of the Reviewer that there are no non-clinical safety signals that would preclude the approval of eslicarbazepine acetate for treatment of partial-onset seizures in adults with epilepsy. However, as a condition of approval, it is the Reviewer's recommendation that the Sponsor should complete, as a post marketing requirement (PMR), a study investigating the mutagenic and clastogenic potential of the main human metabolite, eslicarbazepine, in both the *in vitro* mouse lymphoma mutation assay and the *in vitro* cytogenetic assay in Chinese Hamster Ovary (CHO) cells.

1.1.2. Additional nonclinical comments:

One of the most important characteristics of BIA 2-093 to consider in the non-clinical safety assessment is the distinctly different metabolite profile in rats when compared to humans, dogs, mice and rabbits. Specifically, the Sponsor has demonstrated that rats predominately metabolize BIA 2-093 and the major human metabolite, S-licarbazepine (BIA 2-194), to OXC, a minor human metabolite. Therefore, due to the higher levels of OXC circulating in rats exposed to BIA 2-093, the findings of studies performed with rats are difficult to interpret. Due to this fact, the rat is considered to be an inappropriate animal model for conducting the necessary nonclinical toxicity studies of BIA 2-093. The Sponsor addressed this issue by performing *in vivo* studies in a second rodent species (mouse), which metabolizes BIA 2-093 mainly to BIA 2-194, the major human metabolite.

Although conducting studies in mice allowed for qualification of the main human metabolite, BIA 2-194, in the *in vivo* nonclinical studies, the Sponsor did not address the relevance of using rat hepatic S9 fractions as a biotransformation system in the *in vitro* genotoxicity studies. In addition, the Sponsor did not characterize the metabolites of BIA 2-093 produced by the rat liver S9 fraction as requested by DNP (IND 67466; May Proceed Letter dated 1/26/2007). The Sponsor did, however, measure BIA 2-194 in an *in vitro* metabolism assay where BIA 2-093 was incubated in the presence and absence of rat liver microsomes. In this microsomal metabolism study (093-526), the Sponsor did demonstrate that BIA 2-194 is produced by rat liver microsomes but is detected at levels 20% lower than in the presence of human liver microsomes. Furthermore, OXC levels are 2 fold higher in rat liver microsomes when compared to human liver microsomes. It is important to remember that hepatic S9 fractions, which were used in the *in vitro* genotoxicity assays, contain both cytosolic and microsomal drug metabolizing enzymes. Therefore, the resulting metabolic profile obtained with rat hepatic S9 fractions may differ from the profile obtained with rat hepatic microsomes. Since the Sponsor did not determine which metabolites are produced in the presence of the rat liver S9 fraction, it is

impossible for the Reviewer to determine if these assays contained sufficient levels of the major human metabolite, BIA 2-194. The Sponsor does address this issue in the bacterial reverse mutation assay by adding BIA 2-194 directly to the assay mixture. The results of this analysis were negative. The Sponsor does not, however, address this deficiency in the *in vitro* mammalian mutation assay or the *in vitro* chromosomal aberration assays. In both of these assays, the Sponsor employed rat liver S9 fractions as the biotransformation system, did not test the concentration of BIA 2-194 in the assay mixture and did not add BIA 2-194 directly to the assay. Due to the lack of information regarding the concentrations of BIA 2-194 produced in these assays, the genotoxic potential of BIA 2-093 is considered by the Reviewer to be inadequately characterized in the full battery of genotoxicity assays. The Reviewer, therefore, recommends that the Sponsor be required to conduct an *in vitro* mammalian mutagenicity assay and an *in vitro* chromosomal aberration assay on the main human metabolite, BIA 2-194 (eslicarbazepine).

1.1.3. Draft Labeling: The labeling provided below (in italics) is the Reviewer's draft recommendation for labeling. Dr. Ed Fisher (CDER/DNP) has been consulted by the Reviewer to comment upon the pregnancy section of the label.

Highlights of Prescribing Information
A) Indications and Usage:

STEDESA is an anti-epileptic agent indicated for adjunctive therapy in the treatment of partial-onset seizures in adults with epilepsy (1.1)

B) Use in Specific Populations:

Pregnancy: Based on animal data, may cause fetal harm.

1.1 Indications and Usage:

STEDESA (eslicarbazepine acetate) is an anti-epileptic agent indicated for adjunctive therapy in the treatment of partial-onset seizures in adults with epilepsy.

8.1 Pregnancy

Pregnancy Category C. STEDESA has been shown to be teratogenic in both mice and rabbits and embryocidal in mice when administered to pregnant animals at clinically relevant doses. There are no adequate and well-controlled studies in pregnant women. STEDESA should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Administration of eslicarbazepine acetate (oral doses of 150 to 650 mg/kg) to pregnant mice throughout the period of organogenesis was associated with fetal malformations (cleft palate), decreased number of implantations and decreased number of live fetuses at all doses. In the absence of a no-effect dose for these toxicities, the lowest-effect dose (150 mg/kg) is 0.6 fold of the maximum recommended human dose (MRHD) of 1200

mg/day on a body surface area (mg/m^2) basis. Exencephaly and delayed sexual maturity characterized by delayed vaginal perforation and delayed preputial opening were observed after in utero exposure at a no-effect dose (150 mg/kg) that is 0.6 fold the MRHD on a mg/m^2 basis. Delayed ossification and delayed eye and ear opening in offspring exposed in utero occurred at maternally toxic doses (650 mg/kg) that are 2.6 the MRHD on a mg/m^2 basis.

In rabbits, oral dosing of pregnant females with eslicarbazepine acetate (40 to 320 mg/kg) during organogenesis was associated with increased fetal incidence of irregular ridging of the palate and increased number of presacral vertebrae (due to an increase in the number of thoracic vertebrae) at all doses. In the absence of a no-effect dose for these developmental abnormalities, the lowest-effect dose (40 mg/kg) is 0.65 fold the MRHD on a mg/m^2 basis. Delayed ossification of fetuses exposed in utero occurred at maternally toxic doses (320 mg/kg) that are 5.2 fold the MRHD on a mg/m^2 basis.

8.2 Labor/Delivery

The effect of STEDESA on labor and delivery in humans has not been evaluated.

8.3 Nursing Mothers

Because of the potential for tumorigenicity shown for eslicarbazepine acetate in a mouse study, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

8.4 Pediatric Use

Safety and effectiveness in patients below 18 years of age have not been established.

12.1 Mechanism of Action

The precise mechanism(s) by which eslicarbazepine acetate exerts its antiepileptic action is (are) unknown. Eslicarbazepine acetate is known to be metabolized mainly to eslicarbazepine but its minor metabolites, R-licarbazepine and oxcarbazepine, are also produced in vivo. In vitro electrophysiology studies suggest that eslicarbazepine, R-licarbazepine and oxcarbazepine, the latter being the most potent of the three metabolites, antagonize both the voltage-gated sodium channels in the inactivated state and voltage-gated calcium channels.

13. Non-Clinical

Carcinogenesis, Mutagenesis, Impairment of Fertility

In a two-year carcinogenicity study in mice, eslicarbazepine acetate was administered orally at doses of 100, 250, and 600 mg/kg/day. An increase in the incidence of hepatocellular adenomas and a dose-related increase in the incidence of hepatocellular

carcinomas were observed in males at eslicarbazepine doses of ≥ 250 mg/kg (≥ 1.0 times the MRHD, on a mg/m^2 basis). An increase in the incidence of hepatocellular adenomas and carcinomas was observed in female mice at doses of 600 mg/kg/day (2.3 times the MRHD, on a mg/m^2 basis).

In in vitro genotoxicity studies, eslicarbazepine acetate and the human metabolites eslicarbazepine, R-licarbazepine and oxcarbazepine were not mutagenic in the Ames test. Eslicarbazepine acetate was weakly mutagenic in the mouse lymphoma L5178Y cell mutation test. Eslicarbazepine acetate was clastogenic in the Chinese hamster ovary (CHO) cell cytogenetic test, with chromatid deletion being the predominant finding, but was not clastogenic in the chromosomal aberration assay in human peripheral blood lymphocytes. In vivo, eslicarbazepine acetate was not clastogenic in the mouse micronucleus test and did not induce DNA repair (as measured by unscheduled DNA synthesis) in the mouse liver.

In a fertility study in mice, oral dosing of males and females prior to and during mating and early gestation with eslicarbazepine acetate (150, 350, and 650 mg/kg/day) was associated with a dose-related decrease in the number of implantations and number of live embryos, in all dose groups. In the absence of a no-effect dose, the lowest-effect dose for this finding in mice (40 mg/kg) is 0.6 times the MRHD, on a mg/m^2 basis.

1.2 Evaluation and discussion of nonclinical findings affecting regulatory decision.

1.2.1 Basis of Recommendation:

The Reviewer's recommendation for approval is primarily based on the fact that the non-clinical toxicity profile of eslicarbazepine acetate (BIA 2-093) does not differ substantially from what is described in the labeling of FDA-approved antiepileptics, including those of a similar structure, such as oxcarbazepine (OXC; Trileptal®) and carbamazepine (CBZ; Tegretol®). Furthermore, NDA 22416, with the exception of the deficiency discussed in Section 1.1.2 of this review, contains reports of nonclinical studies which were conducted in a manner consistent with the recommendations made in ICH Guidances M3(R2), S2A, S5A, S1B and S1C. Specifically, the Sponsor satisfactorily qualified the major human metabolite BIA 2-194 by using relevant animal models in adequately designed and conducted pivotal repeat dose toxicology studies, safety pharmacology studies, reproductive and developmental toxicology studies, genotoxicity studies and a carcinogenicity study in mice. Along with relevant species (mouse and dog) being chosen by the Sponsor for the toxicologic assessment of BIA 2-093, the pivotal studies conducted in the non-rodent species were of sufficient length to support the safety of a chronic use medication. The longest general toxicology study in a relevant rodent model was a 13 week repeat dose study conducted in mice. However, the two year mouse carcinogenicity study is of sufficient dosing duration to cover the maximum period of chronic dosing recommended in ICH M3(R2) for a chronic use medication. Although the Sponsor demonstrates that eslicarbazepine is a carcinogen and teratogen in these nonclinical studies, similar findings have been observed in nonclinical studies conducted with other FDA-approved antiepileptics.

In the mouse carcinogenicity study, eslicarbazepine acetate caused hepatic adenomas and hepatocellular carcinomas in male mice exposed to 250 mg/kg and 600 mg/kg (1.0 and 2.3 times the MRHD based on body surface area (mg/m^2)) and in female mice exposed to 600 mg/kg for two years. Although the Sponsor does not provide sufficient evidence to establish the proposed mechanism of action for the eslicarbazepine acetate induced-neoplasia, similar hepatic tumor types have been observed in non-clinical studies performed with other FDA-approved antiepileptics. For example, according to the label for Trileptal®, mice exposed to OXC for two years developed hepatocellular adenomas at doses $\geq$ 0.1 times the MRHD based on dose per body surface area. In the rat carcinogenicity study described on the Trileptal® label, hepatocellular carcinomas and hepatic adenomas were observed at 0.1 and 2.4 times the MRHD based on mg/m^2, respectively. Furthermore, benign interstitial cell tumors of the testes and vaginal and cervical granular cell adenomas and carcinomas, neoplasia that were not observed in eslicarbazepine acetate-exposed mice, were observed in rats exposed to Trileptal® at 2.4 times the MRHD based on dose per body surface area. According to the label for Tegretol®, rats exposed to CBZ for two years exhibited hepatocellular tumors and benign interstitial cell adenomas of the testes when exposed to doses that were 0.6-6.1 times the MRHD based on body surface area. Therefore, even though a robust signal for neoplasia existed with little to no safety margin in mice exposed to eslicarbazepine acetate, this was not out of the ordinary for FDA-approved antiepileptic medications.

Eslicarbazepine acetate exhibited several positive findings in the non-clinical reproductive toxicity studies, such as teratogenicity in mice and rabbits and impairment of fertility in mice. Of greatest concern was the concordant finding in the palate (cleft palate in mice and irregular ridging of the palate in rabbits). The mouse and rabbit palate malformations were observed at doses of eslicarbazepine acetate that were, respectively, 0.6-2.6 and 0.7-5.2 times the MRHD based on body surface area. Similar malformations have been observed in nonclinical studies performed with FDA-approved antiepileptics. The labeling of Trileptal® describes similar malformations occurring in the fetuses of pregnant rats exposed to doses that were 1.2 to 2.4 times the MRHD. Furthermore, Tegretol®, a known human and animal teratogen, which is known to cause craniofacial malformations, was approved as a pregnancy category D medication. Therefore, although the occurrence of teratogenicity in mice exposed to eslicarbazepine acetate is considered a serious safety concern and should be reported in the labeling, the finding of fetal malformations is common in animals exposed to FDA-approved antiepileptics and should not preclude the approval of STEDESA®. The Sponsor also demonstrates in the non-clinical reproductive toxicology studies that eslicarbazepine acetate decreased the number of implantations and the number of live fetuses in mice exposed to 0.6-2.5 times the MRHD. Similar alterations in fertility parameters are described in the labeling of Trileptal® at doses in rats equal to 1.2 and 4 times the MRHD and in rabbits equal to 1.5 the MRHD. Therefore, it is obvious that the alteration of the fertility parameters observed in non-clinical studies with eslicarbazepine acetate were not extraordinary when compared to other FDA-approved antiepileptics. The finding of altered fertility in animals exposed to eslicarbazepine acetate should be addressed in the labeling of STEDESA®.

In summary, the carcinogenic and teratogenic activities of eslicarbazepine acetate that were observed in the non-clinical studies described in NDA 22416 are consistent with

those of other FDA-approved antiepileptics, including those with similar chemical structure, such as Trileptal ® and Tegretol®. Since the findings in animals dosed with eslicarbazepine acetate are consistent with those observed in animals dosed with other FDA-approved antiepileptics, the teratogenic and carcinogenic potential of eslicarbazepine acetate should not preclude its approval as an adjunctive treatment for partial-onset seizures.

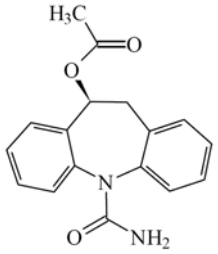
1.2.2 Clinical Implication:

The teratogenicity, fertility and carcinogenicity findings are considered by the Reviewer to have the greatest relevance to the safe human use of BIA 2-093. The main reason for this assessment is the lack of a safety margin for the teratogenicity and fertility findings in animals dosed with BIA 2-093 (Reviewer's Table 10.1). Furthermore, the Sponsor neither demonstrates an acceptable safety margin nor provides a convincing mechanism of action for the hepatic neoplasia (Reviewer's Table 10.1). If approved for marketing, the labeling for STEDESA® should adequately describe the potential risk for these adverse effects in humans.

2. Drug Information

2.1. Drug:

2.1.1. Pharmacological class: Dibenz[b, f] azepine-5-carboxamide derivative.
2.1.2. CAS registry number (optional): 236395-14-5
2.1.3. Generic name: Eslicarbazepine acetate
2.1.4. Code name: SEP-0002093, BIA 2-093, ESL
2.1.5. Chemical name: (S)-10-acetoxy-10,11-dihydro-5H-dibenz[b,f]azepine-5 carboxamide.
2.1.6. Molecular formula/molecular weight: $C_{17}H_{16}N_2O_3$/ 296.32
2.1.7. Structure:



2.1.8. Batches of BIA 2-093 Used in Non-Clinical Studies:

Batch #	Manufactured by	Manufacture Date	Purity
PC96.03.22	BIAL	3/1996	100.7
BN980220	BIAL	2/1998	100.2
BN000222	BIAL	2/2000	99.7
0000012976	(b) (4)	10/2000	99.6
2070-1-1		3/2003	99.7
4674-2-4		12/2003	99.0
19990-2-1		8/2005	99.6
25570-1-3		6/2006	99.3

Reviewer's Table 2.1: Information obtained from NDA 22416 Section 3.2.S.4.4

2.2. Clinical formulation:

2.2.1. Drug formulation: Tablets: 400, 600, 800 mg

2.2.2. Comments on excipients:

All excipients found in this formulation are listed in the "Inactive Ingredient Search for Approved Drug Products Database" and are present in the proposed formulation at levels lower than those found in previously approved drug products.

2.2.3. Comments on impurities/degradants:

The Sponsor stated that the primary stability study and a supportive study are still ongoing. However, no degradants of concern have been observed at the twelve month time point of the stability study. The impurity, (b) (4) was tested in several *in vivo* and *in vitro* studies described in the Toxicology section of

this review. Since a minimal genetic toxicology screen and three-month studies were performed in dogs, rats and mice using preparations of the drug substance containing [redacted] (b) (4) (Batch #00000012976), the criteria for qualification of the impurity have been met. Furthermore, it is stated by the sponsor (page 7 of 2.3.5.4) that [redacted] (b) (4) is present in the final drug product at levels [redacted] (b) (4) similar to those observed in the drug product used in several Phase I and Phase II clinical studies. Other impuritie [redacted] (b) (4) occur in the drug substance at levels below the qualification threshold of 0.15%.

2.3 Proposed clinical population and dosing regimen:

The Sponsor proposes that the dosing regimen in adults with partial-onset seizures should be initially 400 mg once a day for one week. The dose can then be increased at weekly intervals by 400 mg to a maximum daily dose of 1200 mg. A maintenance dose of 800 mg once a day is suggested.

2.4. Regulatory background:

Documentation of the communications between the Sponsor and the FDA can be found in IND 67466 and its associated submissions. A special protocol/carcinogenicity assessment request was submitted on 7/15/03 by Bial Portela and Companhia, S.A. The protocol was assessed by the Executive Carcinogenicity Assessment Committee (ExecCAC) and a response regarding the assessment was provided to the Sponsor on 8/28/03. On July 20, 2007, a waiver of the rat carcinogenicity study for BIA 2-093 was granted. A pre-NDA meeting was held on 1/23/2008. Bial transferred ownership of IND 67466 to Sepracor on 4/10/08. A telephone conference was held on 11/13/2008. The NDA, submitted by Sepracor, was received by CDER on 3/30/09.

Eslicarbazepine acetate has not been previously approved by the FDA. [redacted] (b) (4) However, the European Medicines Agency (EMEA) has issued a "Summary of Positive Opinion" on February 19, 2009 for eslicarbazepine acetate to be marketed in Europe under the trade names Exalief® and Zebinix®. The marketing authorizations for Exalief® and Zebinix® were granted by the EMEA on April 21, 2009.

Previous clinical experience: Eslicarbazepine acetate has only recently become available in the EU (4/2009).

Relevant IND/s, NDA/s, and DMF/s: DMFs: 22026, [redacted] (b) (4) IND: 67466

Interaction/s w/ Agency: A summary of interactions with the FDA is provided in the Sponsor's table below. Details regarding interactions with the FDA are also contained in the Regulatory History section above.

Date	Meeting/Communication
28 Aug 2003	Carcinogenicity Assessment Committee (CAC) response to Special Protocol Assessment regarding mouse carcinogenicity study protocol, and initial response to request for waiver for rat carcinogenicity study
21 July 2006	Pre-IND Meeting
23 Aug 2006	Formal minutes from Pre-IND meeting
20 July 2007	Final response granting waiver for rat carcinogenicity study
23 Jan 2008	Pre-NDA meeting (Type B)
2 Apr 2008	Formal minutes from Pre-NDA meeting – see Attachment 1
9 Apr 2008	Transfer of IND from Bial to Sepracor effective 10 April 2008
14 Aug 2008	Response to outstanding issues from Pre-NDA meeting – see Attachment 2
13 Nov 2008	Type C additional Pre-NDA Meeting (Teleconference)
12 Jan 2009	Formal minutes from Type C Teleconference – see Attachment 3
10 Mar 2009	Telecon identifying that submission of the nonclinical abuse liability data by the Day 120 update will not constitute a filing issue.

Sponsor's Table: Meeting/Communications with the FDA

3. Studies submitted within this submission:

3.1. Studies reviewed within this submission:

Primary Pharmacodynamics:

a) *093-413*: Effects of Oxcarbazepine in the maximal Electroconvulsive Shock Test in the Mouse
b) *093-403*: Bial Substances: Functional Effects on Sodium Currents Recorded from N1E-115 Mouse Neuroblastoma Cells
c) *093-420*: BIA2-194 and BIA2-195: Modulatory Effects on Recombinant GlyRα3 Currents Recorded from Stably Transfected CHO Cells.
d) *093-422*: Oxcarbazepine: Modulatory Effects on Recombinant $GABA_A\alpha1$ Currents Recorded From Stably Transfected LTK Cells.
e) *093-423*: BIA 2-194, BIA 2-195 and Oxcarbazepine: Effect on $GABA_A\alpha2$ Currents Recorded from Stably Transfected LTK Cells
f) *093-424:* BIA 2-194, BIA 2-195 and oxcarbazepine: Effect on $GABA_A\alpha3$ Currents Recorded from Stably Transfected LTK Cells
g) *093-404*: Bial Substances: Effect on $Na_v1.8$ Peak Currents Recorded from Transfected ND7/23
h) *093-418*: BIA2-194 and BIA2-195: Modulatory Effects on Recombinant GABA Currents Recorded from Stably Transfected LTK Cells.
i) *093-410:* Effects of BIA 2-093 in the Maximal Electroconvulsive Shock Test in the Mouse (Oral Administration)
j) *093-400:* BIA2-194 and BIA2-195: Affinities for the Resting and Inactivated States of Endogenous Sodium Channels from N1E-115 Mouse Neuroblastoma Cells.
k) *093-414*: Effect of BIA 2-093, BIA 2-005, BIA 2-194, BIA 2-195 and Oxcarbazepine on the Progression of Corneal Kindling.
l) *093-416*: Oxcarbazepine: Effects on the Endogenous Potassium Channels Recorded from N1E-115 Mouse Neuroblastoma Cells.
m) *093-417:* Oxcarbazepine: Effects on the Endogenous Calcium Channels Recorded from N1E-115 Mouse Neuroblastoma Cells.

n) *093-425*: BIA 2-194, BIA 2-195 and Oxcarbazepine: Effect on $GABA_A\alpha5$ Currents Recorded from Stably Transfected LTK Cells.
o) *093-412*: Effects of BIA 2-195 in the Maximal Electroconvulsive Shock Test in the Mouse (Oral Administration).
p) *093-401*: BIA2-194 and BIA2-195: Affinities for the Resting and Inactivated States of $Na_v1.8$ Sodium Channels from Transfected ND7/23 Neuroblastoma Cells.
q) *093-402*: Effects of BIA 2-093 on Voltage-Dependent Sodium Currents in N1E-115 Cells: Comparison with Carbamazepine.
r) *093-405*: Effect of BIA2-194 and BIA2-195 on $hNa_v1.7$ Peak Currents Recorded from Transfected CHO cells- IC_{50} determination.
s) *093-407*: *In vitro* Pharmacological Profile of BIA 2-093 (Eslicarbazepine acetate), a New Antiepileptic Drug.
t) *093-409*: Follow up Study: Effects of BIA 2-093 in the Maximal Electroconvulsive Shock Test in the Mouse (Oral Administration)
u) *093-411*: Effects of BIA 2-194 in the Maximal Electroconvulsive Shock Test in the Mouse (Oral Administration).
v) *093-419*: BIA2-194 and BIA2-195: Effect on $Ca_v3.2$ (T-type) channels recorded from Stably Transfected HEK 293 Cells.
w) *093-406*: Effect of BIA2-194 and BIA2-195 on $hNa_v1.6$ Peak Currents Recorded from Transfected CHO cells- IC_{50} Determination.
x) *093-408*: *In vivo* Pharmacological Profile of BIA 2-093 (Eslicarbazepine acetate), a New Antiepileptic Drug.

Secondary Pharmacodynamics:
a) *093-415*: Neurosolutions, LTD Report
b) *093-421*: Evaluation of BIA 2-093, BIA 2-194, BIA 2-195 and Oxcarbazepine Using the Formalin (Late Phase) Test in the Mouse (p.o. administration).

Safety Pharmacology:
a) *093-856*: BIA 2-195: Effect on HERG Tail Currents Recorded from Stably Transfected HEK 293 Cells.
b) *093-854*: BIA 2-005: Effect on HERG Tail Currents
c) *093-860:* Evaluation of BIA 2-093 in the Diuresis-Naturesis Test in the Mouse (P.O. Administration)
d) *093-864*: Customized Screening Program Prepared for BIAL
e) *093-850*: Evaluation of BIA 2-093 in the Irwin Test in the Mouse (P.O. Administration)
f) *093-861*: Effects of BIA 2-093 on Renal Function in Saline-Loaded Rats
g) *093-862*: Evaluation of BIA 2-093 in the Gastrointestinal Transit test in the Mouse (P.O. Administration)
h) *093-865*: Customized Screening Program Prepared for BIAL
i) *093-858*: Assessment of Cardiovascular Risk of BIA 2-093 in the Conscious Dog Monitored by Telemetry (P.O. Administration)
j) *093-853*: BIA 2-093: Effect on HERG Tail Currents Recorded from Stably Transfected HEK 293 Cells.
k) *093-863*: Effect of BIA 2-093 on the gastrointestinal Transit of a Charcoal Meal in Rats

l) *093-855*: BIA 2-194: Effect on HERG tail Currents Recorded from Stably Transfected HEK 293 Cells
m) *093-849:* Customized Profile Screening Program for BIAL
n) *093-851*: Effects of BIA 2-093 in the Irwin Test in Rats
o) *093-852*: Evaluation of BIA 2-093 on Respiratory Function Following P.O. Administration in the Mouse (Whole Body Plethysmography).
p) *093-859*: Effects of BIA 2-093 on General Haemodynamics and Respiration Rat in Anesthetized Beagle Dogs.
q) *093-857*: Effects of BIA 2-093, BIA 2-005 and Oxcarbazepine on Action potential Parameters in Dog isolated cardiac Purkinje Fibers

Analytical Methods and Validation Reports:

a) *093-502*: Assessment of the Stability of BIA 2-093 in Oral Dosing Formulations of 0.5% Hydroxypropylmethylcellulose by HPLC with Ultra-Violet Detection in Support of Preclinical Studies.
b) *093-503:* Analysis of BIA 2-194 in 0.5% w/v HPMC in Support of Preclinical Studies.
c) *093-513:* Determination of BIA 2-093, BIA 2-194, BIA 2-195 and Oxcarbazepine in Dog Plasma by Chiral LC-MS/MS.
d) *093-505:* Validation of an Analytical Procedure for the Determination of BIA 2-093 in Dosing Formulations of 0.5% Hydroxypropylmethylcellulose by HPLC with UV Detection in Support of Preclinical Studies.
e) *093-509:* Validation of a Method for the Determination of BIA 2-093 and Its Metabolites, BIA 2-194, BIA 2-195, and Oxcarbazepine, in Mouse Plasma by LC-MS/MS.
f) *093-501:* Determination of BIA 2-093in Dosing Formulations of 0.5% Hydroxypropylmethylcellulose by HPLC with UV Detection in Support of Preclinical Studies.
g) *093-504:* Determination of BIA 2-195 in Dose Solutions (0.5% Hydroxypropylmethylcellulose w/v) in Support of Preclinical Studies by HPLC with UV Detection.
h) *093-500:* HPLC Purity and Assay
i) *093-506:* Validation of an Analytical Procedure for the Determination of BIA 2-194 in Dose Solutions in Support of a Preclinical Study by HPLC with UV Detection.
j) *093-508:* Partial Validation of an Analytical Procedure for the Determination of BIA 2-093 and its Metabolites, BIA 2-005 and Oxcarbazepine, in Dog, Rat and Mouse Plasma Using Solid Phase Extraction Followed by HPLC with Mass Spectrometric Detection.
k) *093-511:* Validation of an HPLC Method with UV Detection for the Quantification of BIA 2-093, the 10,11-Dihydro Metabolite and Oxcarbazepine in Rat and Dog Plasma.
l) *093-507:* Validation of an Analytical Procedure for the Determination of BIA 2-195 in Dose Solutions (0.5% Hydroxypropylmethylcellulose w/v) in Support of Preclinical Studies by HPLC with UV Detection.
m) *093-510:* Determination of BIA 2-194 and BIA 2-195 in Rat Plasma by Chiral LC-MS/MS.
n) *093-512:* Validation of a Method for the Determination of BIA 2-093 and Its Metabolites BIA 2-194, BIA 2-195 and Oxcarbazepine, in Rabbit Plasma by LC-MS/MS.

Pharmacokinetics:

a) *093-514*: The Disposition of Total Radioactivity in the Mouse Following Single Oral and Single Intravenous Administration of [^{14}C] BIA 2-093
b) *093-518*: The Disposition of Total Radioactivity in the Dog Following Single Oral and Single Intravenous Administration of [^{14}C] BIA 2-093
c) *093-519*: Transport of BIA 2-093 and Metabolites in Caco-2 Cells.
d) *093-516*: The Disposition of Total Radioactivity in the Rat Following Single Oral and Single Intravenous Administration of [^{14}C] BIA 2-093
e) *093-517:* Toxicokinetic and Metabolism Study in the Pregnant Rabbit
f) *093-520:* The Tissue Distribution of Total radioactivity in the Mouse Following Single Oral Administration of [^{14}C] BIA 2-093
g) *093-521*: Brain Pharmacokinetics of BIA 2-194 and BIA 2-195: Impact of Multidrug Transporter Inhibition
h) *093-525:* Determination of the *In Vitro* Plasma Protein Binding and Blood Cell Binding of [^{14}C] BIA 2-194 in Rat and Man
i) *093-523*: Evaluation of Drug Concentrations in Plasma and Cerebral Spinal Fluid of Cynomolgus Monkeys Following Treatment with Eslicarbazpine Acetate and Oxcarbazepine.
j) *093-524*: Determination of the *In Vitro* Binding of [^{14}C] BIA 2-093 to Plasma Proteins and Blood Cells of Animals and Man
k) *093-522*: Whole Body Phosphor Imaging in the Male Rat Following Single Oral Administration of [^{14}C] BIA 2-093.
l) *093-526: In Vitro* Metabolic Profiling Study with [^{14}C] BIA 2-093 in Rat, Dog, Primate and Human Liver Microsomes.
m) *093-527*: Investigation of Milk Transfer in the Mouse Following Single Oral Administration of [^{14}C] BIA 2-093.
n) *093-529:* Determination of the *In Vitro* Plasma Protein Binding Displacement of BIA 2-194
o) *093-533:*Incubation of BIA 2-194 with Fresh Human hepatocytes to Assess Potential Induction of Hepatic Enzymes
p) *093-528:* Investigation of the Potential Inhibitory Effect of a Range of Anti-Epileptic Drugs on the Metabolism of [^{14}C] BIA 2-093.
q) *093-534:* Incubation of BIA 2-194, BIA 2-195 and Oxcarbazepine with Fresh Human Hepatocytes to Assess Potential Induction of Hepatic CYP3A4.
r) *093-530:* An *In Vitro* Study to Examine the Effect of BIA 2-194 on Human Hepatic Enzyme Activity
s) *093-531*: An *In Vitro* Study to Examine the Effect of BIA 2-194, BIA 2-195 and Oxcarbazepine on Human Hepatic CYPC19 Enzyme Activity
t) *093-532:* An *In Vitro* Study to Examine the Effect of BIA 2-194, BIA 2-195 and Oxcarbazepine on Human Hepatic CYPC19 Enzyme Activity

Toxicology:

Single Dose:

a) *093-803:* Estimation of the Acute Oral Lethal Dose in the Rat
b) *093-801*: Estimation of the Acute Oral Lethal Dose in the Mouse
c) *093-804*: Estimation of the Acute IV Lethal Dose in the Rat

d) *093-802:* Estimation of the Acute IV Lethal Dose in the Mouse

Repeat Dose:
Rat, Subchronic
a) *093-807*: 2 Week Oral (Gavage) Dose Range Finding Study in the Rat
b) *093-811*: 4 Week Preliminary Toxicity Study by Oral (Gavage) Administration to Rats
c) *093-808*: Four Week Oral (Gavage) Repeat Dose Toxicity Study in the Rat
d) *093-809*: Three Month Oral (Gavage) Repeat Dose Toxicity Study in the Rat with a Four Week Recovery Period
e) *093-813*: Three Month Oral Repeat Dose Comparative Toxicity Study in the Han Wistar Rat

Rat, Chronic
a) *093-812*: 13 Week Oral (Gavage) Dose Range Finding Study in the Rat
b) *093-810*: 26 Week Oral (Gavage) Toxicity Study in the Rat

Dog, Subchronic
a) *093-816*: Four Week Oral (Capsule) Repeat Dose Toxicity Study in the Beagle Dog
b) *093-815:* Oral Maximum Tolerated Dose (MTD) and 14 Day Repeat Dose Study in the Beagle Dog
c) *093-817*: Three Month Oral (Capsule) Repeat Dose Toxicity Study in the Beagle Dog with a Four Week Recovery Period

Dog, Chronic
a) *093-818:* Six Month Oral (Capsule) Repeat Dose Toxicity Study in the Beagle Dog
b) 093-819: Twelve Month Oral (Capsule) Repeat Dose Toxicity Study in the Beagle Dog

Mouse, Subchronic
a) *093-805:* 4 Week Oral (Gavage) Dose Range Finding Study in the Mouse
b) *093-806:* Thirteen Week Oral (Gavage) Dose Range Finding Study in the Mouse

Rabbit, Dose ranging
a) *093-814:* Oral Gavage Maximum Tolerated Dose Study in the Rabbit

Genotoxicity:
In vitro:
a) *093-821*: BIA 2-194: Testing for Mutagenic Activity with Salmonella typhimurium TA 1535, TA 100, TA 1537 and TA 98 and Escherichia coli WP2uvrA
b) *093-825*: *In Vitro* Mammalian Cell Cytogenetic Test: Chinese Hamster Ovary Cells.
c) *093-823*: Oxcarbazepine: Testing for Mutagenic Activity with Salmonella typhimurium TA 1535, TA 100, TA 1537 and TA 98 and Escherichia coli WP2uvrA
d) *093-824:* Mouse Lymphoma Cell Mutation Assay
e) *093-826*: *In vitro* Mammalian Cell Cytogenetic Test: Chinese Hamster Ovary Cells.
f) *093-827:*BIA 2-093 Chromosomal Aberrations Assay with Human Peripheral Lymphocytes *in vitro*
g) *093-820:* Bacterial Reverse Mutation Test
h) *093-822:* BIA 2-195 Testing for Mutagenic Activity with Salmonella typhimurium TA 1535, TA 100, TA 1537 and TA 98 and Escherichia coli WP2uvrA.

In vivo:

a) *093-829*: BIA 2-093: *In vivo* Mouse Liver Unscheduled DNA Synthesis Assay
b) *093-828:* Mouse Micronucleus Test

Carcinogenicity:

a) *09-830*: Data Definitions Table; XPT File; and Report entitled "104 Week Oral (Gavage) Carcinogenicity Study in the Mouse."

Reproductive and Developmental Toxicity:

a) *093-831*: Oral (Gavage) Fertility and Early Embryonic Developmental Study in the Mouse
b) *093-833:* Oral (Gavage) Fertility and Early Embryonic Developmental Study in the Rat
c) *093-832:* Oral (Gavage) General Reproductive Performance Dose Ranging Study in the Rat
d) *093-837*: Oral (Gavage) Developmental Toxicity Study in the Rabbit
e) *093-847*: Toxicokinetic Study in the Pregnant Mouse
f) *093-834:* Oral (Gavage) Developmental Toxicity Study in the Mouse
g) *093-835*: Oral (Gavage) Developmental Toxicity Study in the Rat
h) *093-836:* Oral (Gavage) Developmental Toxicity Dose Range Finding Study in the Rabbit
i) *093-839:* Oral (Gavage) Pre and Post-natal Developmental Toxicity Study in the Mouse
j) *093-838*: Oral (Gavage) Pre and Post natal Developmental Toxicity Dose Ranging Finding Study in the Mouse
k) *093-840:* Oral (Gavage) Pre- and Post-natal Developmental Toxicity Study in the Rat

Other Toxicity Studies:

Impurities:

a) *093-844:* (b) (4) Acute Intravenous Toxicity test in Rats
b) *093-843*: (b) (4) Acute Oral Toxicity Test in Rats
c) *093-846:* (b) (4) Chromosomal Aberrations Assay with Human Peripheral Lymphocyte Cultures *In Vitro*
d) *093-842:* (b) (4) Acute Intravenous Toxicity Test in Mice
e) *093-845:* (b) (4) Testing for Mutagenic Activity with Salmonella typhimurium TA 1535, TA 100, TA 1537 and TA 98 and Escherichia coli WP2uvrA
f) *093-841*: (b) (4) Acute Oral Toxicity Test in Mice

3.2. Studies not reviewed within this submission:

a) Non Clinical Abuse Potential Summary
b) *093-848*: Drug Dependency- Consideration of Risk
c) *093-874*: *In Vitro* Pharmacology: Binding Assays-Study of BIA 2-093, Eslicarbazepine, R-Licarbazepine, and Oxcarbazepine.

3.3. Additional Studies Submitted After CDER Stamp Date:

Studies Submitted on 7/31/09:

Pharmacology:

a) *093-426*: Effects of Oxcarbazepine, Carbamazepine, and Licarbazepine on GABA-Induced Currents From $GABA_A$ Receptors Expressed in Xenopus Oocytes.

b) *093-427:* BIA 2-194/BIA 2-195: Effects on the endogenous calcium channels recorded from N1E-115 Mouse Neuroblastoma Cells.

c) *093-428*: BIA2-194, BIA 2-195 and Oxcarbazepine: Affinities for the resting and inactivated states of $Na_V1.3$ sodium channels from transiently transfected CHO cells.

d) *093-429*: Effect of Oxcarbazepine on $hNa_V1.3$ Peak Currents recorded from Transfected CHO cells-IC_{50} determination.

e) *093-430:* Effect of Oxcarbazepine on $hNa_V1.7$ Peak Currents recorded from Transfected CHO cells-IC_{50} determination.

f) *093-431:* BIA2-194, BIA 2-195 and Oxcarbazepine: Affinities for the resting and inactivated states of $Na_V1.7$ sodium channels from transiently transfected CHO cells.

g) *093-432*: Oxcarbazepine: Affinities for the resting and inactivated states of $Na_V1.8$ sodium channels from transiently transfected ND7/23 Neuroblastoma Cells.

h) *093-433*: Evaluation of BIA 2-093 for Potential Anticonvulsant Activity in the 6 Hz Psychomotor Seizure Model in the Mouse.

i) *093-434:* Follow Up Study: Evaluation of BIA 2-093 for Potential Anticonvulsant Activity in the 6 Hz Psychomotor Seizure Model in the Mouse (P.O. Administration).

j) *093-872: In Vitro* Pharmacology: Brain Benzodiazepine Receptor Binding Assay-Study of BIA 2-093, Eslicarbazepine, (R)-Licarbazepine and Oxcarbazepine.

Safety Pharmacology:

a) *093-873:* Evaluation of BIA 2-093, BIA 2-194, BIA 2-195 and Oxcarbazepine for Cognition Impairing Effects Using the Passive Avoidance Test in the Mouse (I.P. Administration).

Pharmacokinetics:

a) *093-535:* Apparent Permeability of BIA2-093 in Caco-2 cells.
b) *093-536:* Investigation of Placental Transfer in the Mouse Following Single Oral Administration of [^{14}C] BIA 2-093.
c) *093-537:* Investigation of Plasma Protein and Blood Cell Binding of [14C] BIA 2-194 in Mouse Using Ultrafiltration.
d) *093-538:* Eslicarbazepine Acetate, Oxcarbazepine and Carbamazepine Liver CYP 450 Induction in Mouse.

Toxicology:

a) *093-868*: BIA 2-194: Local Lymph Node Assay in the Mouse
b) *093-869*: BIA 2-195: Local Lymph Node Assay in the Mouse
c) *093-870*: Oxcarbazepine: Local Lymph Node Assay in the Mouse

3.4. Previous reviews referenced: "Rat Carcinogenicity Study Waiver Request" (Dr. Ed Fisher, dated April 10, 2007).

4. Pharmacology

4.1 Primary pharmacology:

Reviewer's Summary:

- **BIA 2-194 and BIA 2-195 antagonize, *in vitro*, the Ca_V 3.2 voltage-gated calcium channel, Na_V 1.7, Na_V 1.8, Na_V 1.6 voltage-gated sodium channels and the GlyRα3 glycine receptor.**
- **BIA 2-093 and its metabolites exhibit greater affinity for voltage-gated sodium channels that are in the inactivated state.**
- **BIA 2-093, BIA 2-194, BIA 2-195 are not as potent as OXC at antagonism of sodium and calcium channels.**
- **BIA 2-093 is effective *in vivo* at ameliorating both chemically and electrically-induced seizures.**

Mechanism of Action Studies: To determine the mechanism of action of eslicarbazepine acetate (BIA 2-093), the Sponsor performed *in vitro* electrophysiology studies, *in vitro* receptor interaction studies and *in vivo* seizure mitigation studies. The main result of the *in vitro* electrophysiology studies demonstrates that the active metabolites of BIA 2-093, eslicarbazepine (BIA 2-194) and (R)-licarbazepine (BIA 2-195), are antagonists of GlyRα3, Na_V 1.6, Na_V 1.7, Na_V 1.8 and Ca_V 3.2 (Reviewer's Table 4.1). BIA 2-194 exhibits its greatest potency at the high and low affinity sites of the Ca_V 3.2 voltage-gated calcium channel followed by Na_V 1.7, Na_V 1.8, Na_V 1.6 and GlyRα3, respectively. The minor human metabolite, BIA 2-195, exhibits its greatest potency at the high affinity site of the Ca_V 3.2 voltage-gated calcium channel followed by Na_V 1.7, the low affinity site of Ca_V 3.2, Na_V 1.8, GlyRα3 and Na_V 1.6, respectively. Neither of the metabolites was demonstrated to function as an antagonist of the $GABA_A$ α2, α3, or α5 receptors at concentrations of greater than 1 mM, *in vitro*. The Sponsor confirmed the lack of effect on $GABA_AR$ containing α1, α2, α3, or α5 subunits in electrophysiological experiments using *Xenopus* oocytes (Study 093-426) and by demonstrating the inability of BIA 2-093 and its metabolites to markedly displace flunitrazepam from rat cerebral cortex membrane preparations (093-872). Overall, these studies suggest that, at lower concentrations, the main target of BIA 2-093 metabolites may be voltage gated calcium channels and at higher concentrations these metabolites may target the voltage gated sodium channels. Neither of the metabolites (BIA 2-194 and BIA 2-195) is as potent as oxcarbazepine at antagonizing the voltage gated sodium and calcium channels.

Target	Study	BIA 2-093 (BIA 2-093)	Eslicarbazepine (BIA 2-194)	(R)- Licarbazepine (BIA 2-195)	Oxcarbazepine (OXC)
GlyRα3 (IC_{50}, μM)	093-420	----	~844	~821	----
$GABA_A$α1 (EC_{50}, μM)	093-422	----	----	----	> 1000
$GABA_A$α2 (EC_{50}, μM)	093-423	----	> 1000	> 1000	> 1000
$GABA_A$α3 (EC_{50}, μM)	093-424	----	> 1000	> 1000	> 1000
$GABA_A$α5 (EC_{50}, μM)	093-425	----	> 1000	> 1000	> 1000
Na_V 1.8 at -80mV (IC_{50}, μM)	093-404	443	728	732	438
Na_V 1.7 at -80 mV (IC_{50}, μM)	093-405 093-430	----	477	244	44.1
Na_V 1.6 at -80mV (IC_{50}, μM)	093-406	----	1072	1724	----
Na_V 1.3 at -80mV (IC_{50}, μM)	093-429	----	----	----	549
Ca_V 3.2, T-type (IC_{50}, μM)	093-419	----	3.1 & 166	4.6 & 560	----

Reviewer's Table 4.1- Metabolites of BIA 2-093 Inhibit Glycine, Voltage-Gated Sodium and Voltage-Gated Calcium Channels In Vitro. The IC_{50} or EC_{50} for each compound is provided above. $Ca_V3.2$ voltage gated calcium channels exhibited a biphasic response suggesting the existence of a high and low affinity site. The test articles were bath perfused over transfected cell cultures expressing the receptors listed above. Using whole-cell recordings with a holding potential of -80mV, the effect of the test articles on channel activity was determined. Voltage-gated sodium channels ($Na_V1.6$-1.8) were activated by depolarizing cells to -10mV-0mV, depending on the channel being tested. Voltage-gated calcium channels ($Ca_V3.2$) were activated by depolarizing cells to -25 mV. $GABA_A$ channels were activated by bath perfusing 1-2 μM GABA and GlyR was activated by bath perfusion of 150μM glycine. The systems were validated by using positive controls (Valproate in $Ca_V3.2$ study, TTX in $Na_V1.6$ and 1.7 studies, Bicuculine in $GABA_A$ studies, Tetracaine in $Na_V1.8$ studies, and Strychnine for GlyR studies). ---- = assay not performed. These studies were not GLP-compliant.

The Sponsor also demonstrated that the potency of BIA 2-093 and its metabolites at inhibiting the action of voltage-gated sodium channels increases at lower holding potentials. Specifically, the efficacy of BIA 2-093, BIA 2-194, BIA 2-195 and OXC at inhibiting the response of voltage-gated sodium channels increases from two to six fold when the holding potential is lowered from -80mV to -60 mV (Reviewer Table 4.2). Furthermore, BIA 2-093 and its metabolites do not exhibit the same potential-dependent activity towards GABA and potassium currents. This effect was not tested with respect to voltage-dependent calcium channels, the target demonstrated to be the most sensitive to BIA 2-093 metabolites. Overall, these results suggest that BIA 2-093 and its metabolites exhibit greater affinity for voltage-gated sodium channels that are in the inactivated state compared to those in the resting state.

Target	Study	BIA 2-093 (BIA 2-093)	Eslicarbazepine (BIA 2-194)	(R)- Licarbazepine (BIA 2-195)	Oxcarbazepine (OXC)
Sodium Currents N1E-115 cells at -80 mV (IC_{50}, µM)	093-403	740	3105	1014	805
Sodium Currents N1E-115 cells at -60 mV (IC_{50}, µM)	093-403	207	562	530	172
GABA Currents LTK cells at -80mV (EC_{50}, µM)	093-418	----	> 1000	> 1000	----
Potassium Channels N1E-115 (IC_{50}, µM)	093-416	----	----	----	> 500
Calcium Channels N1E-115 (IC_{50}, µM)	093-417; 093-427	----	345	337	135

Reviewer's Table 4.2- Antagonism of the Voltage-Gated Sodium Channels by BIA 2-093 and its Metabolites is Dependent on Holding Potential. The IC_{50} or EC_{50} for each compound is provided above. The test articles were bath perfused over transfected cell cultures expressing the receptors listed above. Using whole-cell recordings with a holding potential of -100mV, -80mV or -60 mV, the effect of the test articles on voltage-gated sodium channel activity was determined. These studies were not GLP-compliant. GABA channels were activated by bath perfusion of 1µM GABA. Potassium channels were activated by adjusting the holding potential to +60 mV. Calcium channels were activated by adjusting the holding potential to -10 mV. Positive controls were 4-aminopyridine (potassium channel antagonist), Midazolam (GABA channel agonist), $CoCl_2$ (calcium channel antagonist). ---- = assay not performed.

To support the observation that the potency of BIA 2-093 and its metabolites increase as cells are depolarized, the Sponsor performed two studies which demonstrate that the affinity of these compounds is greater for the voltage-gated sodium channel in the inactivated state compared to the resting state (Reviewer Table 4.3). Further examining this issue, the Sponsor demonstrated in study # 093-402 that this phenomenon also exists for carbamazepine and BIA 2-093. However, it is questionable if BIA 2-093 would exhibit this activity *in vivo* since this compound is rapidly hydrolyzed to the active metabolites BIA 2-194 and BIA 2-195. These studies suggest that the antagonistic activity of the active metabolites of BIA 2-093 originates from their selectivity for the inactivated state of the voltage-gated sodium channels. However, OXC exhibits the greatest affinity for the inactivated sodium channel subunits when compared to BIA 2-194 and BIA 2-195.

In a study examining neurotransmitter release from striatal slices (Study 093-407), the Sponsor demonstrated that BIA 2-093 exhibits a dose-dependent inhibition of neurotransmitter release secondary to its antagonism of voltage-gated sodium and calcium channels. This observation suggests that this compound may be effective at decreasing neuronal activity.

Study	Study #	K_i BIA 2-194	K_i BIA 2-195	K_i OXC	BIA 2-194 Kr/Ki ratio	BIA 2-195 Kr/Ki ratio	OXC Kr/Ki Ratio
Affinity for Na Channel in N1E-115 Cells	093-400	245 µM	284 µM	-----	85.7	13.3	-----
Affinity for $Na_V1.3$ in CHO Cells	093-428	165 µM	96.5 µM	29.8 µM	29.1	56.3	62.8
Affinity for $Na_V1.7$ in CHO Cells	093-431	79.8 µM	117.6 µM	26.7 µM	31.7	20.3	560.1
Affinity for $Na_V1.8$ in ND/73 Cells	093-401; 093-432	654 µM	1.3 mM	121.7 µM	2.9	3.7	11.1

Reviewer's Table 4.3-Metabolites of BIA 2-093 Exhibit a Greater Affinity for the Inactive State of the Voltage-Gated Sodium Channel (VGSC) than the Resting State. K_r=Affinity for the resting state of the VGSC, K_i=Affinity for the inactive state of the VGSC. BIA 2-194=Eslicarbazepine, BIA 2-195= (R)-Licarbazepine, OXC= Oxcarbazepine. Studies were not GLP-compliant. K_r and K_i are not shown for OXC. ---- = assay not performed.

BIA 2-093 (administered intraperitoneally) effectively ameliorated the seizures resulting from exposure to the GABA antagonists pentylenetetrazol (metrazole), bicuculine and picrotoxin, in rats (Reviewer's Table 4.4). Although not as potent as in the GABA antagonist trials, BIA 2-093 also inhibited the generation of seizures by 4-aminopyridine (4-AP). However, intraperitoneal injections of BIA 2-093 were not effective at blocking the seizures generated by the glutamate agonists (NMDA, kainate) and the glycine receptor antagonist, strychnine.

Agent	BIA 2-093 (mg/kg)	CBZ (mg/kg)	OXC (mg/kg)
Metrazole	15-30	>60	>60
Bicuculine	15-45	5-15	60-90
Picrotoxin	20-30	5-15	5-15
4-AP	90	~15	15-30
NMDA	NE	NE	NE
Kainate	NE	NE	NE
Strychnine	NE	NE	NE

Reviewer's Table 4.4- BIA 2-093 Inhibits the Generation of Chemically-Induced Seizures (Study #093-408). The doses of BIA 2-093, carbamazepine (CBZ) and oxcarbazepine (OXC) provided in this table resulted in a 50% decrease in seizure generation. NE= no efficacy observed up to 200 mg/kg. 4-AP=4-aminopyridine; NMDA= N-methyl-D-Aspartate.

BIA 2-093 was effective at increasing the number of stimulations required to induce seizures in the mouse electroconvulsive shock test (MEST; Study 093-414 & 093-408). BIA 2-093 exhibited the greatest potency in the MEST compared to BIA 2-194 and BIA 2-195. Examination of blood levels of BIA 2-093 and its metabolites in the MEST suggest that the metabolites (BIA 2-194 and BIA 2-195), and not the parent, are the active species (Reviewer's Table 4.5). This is due to the fact that the parent compound cannot be detected *in vivo* during the MEST. Overall, BIA 2-093 is effective *in vivo* for ameliorating both chemically- and electrically-induced seizures. BIA 2-093 was also shown to be effective in the Mouse 6 Hz Psychomotor Seizure Model (Studies 093-433; 093-434).

Subject	Study #	BIA 2-093 (BIA 2-093)	Eslicarbazepine (BIA 2-194)	(R)- Licarbazepine (BIA 2-195)	Oxcarbazepine (OXC)
Mouse MEST w/ BIA 2-093	093-409	0 ng/ml	5651 ng/ml	0 ng/ml	1162 ng/ml
Mouse MEST w/BIA 2-093	093-410	0 ng/ml	2205 ng/ml	0 ng/ml	251 ng/ml
Mouse MEST w/ BIA 2-194	093-411	0 ng/ml	7464 ng/ml	75 ng/ml	1050 ng/ml
Mouse MEST w/ BIA 2-195	093-412	0 ng/ml	159 ng/ml	10640 ng/ml	1215 ng/ml
Mouse MEST w/ OXC	093-413	0 ng/ml	231 ng/ml	0 ng/ml	3740 ng/ml

Reviewer's Table 4.5- BIA 2-093 is Metabolized to BIA 2-194, BIA 2-195 and OXC in Mice. The plasma concentrations shown are those that are detected at the lowest effective oral dose in the MEST (25 mg/kg BIA 2-093-Study 093-409;10 mg/kg BIA 2-093- Study 093-410; 25 mg/kg BIA 2-194; 50 mg/kg BIA 2-195 and 25 mg/kg OXC).

In summary, the Sponsor has demonstrated that both BIA 2-194 and BIA 2-195 are effective at preventing seizure or raising the threshold for seizure in rodent models of chemically and electrically-induced seizures. This anti-seizure activity in animal models may be due to the demonstrated ability of these compounds to both antagonize voltage-gated sodium and calcium channels *in vitro* and to decrease neurotransmitter release in *ex vivo* models of evoked release. Finally, this compound and its metabolites exhibit high affinity interaction with sodium channels in the inactive and not in the resting state. Overall, the pharmacodynamic properties of this compound suggest that it may have a therapeutic effect in patients with epilepsy.

4.2 Secondary pharmacology

Reviewer's Summary:

- **BIA 2-093 and its metabolites exhibit a robust analgesic effect in rodent models of neuropathic pain.**
- **The *in vitro* binding activity of BIA 2-093 (Adenosine A1, Dopamine D1, $GABA_A\alpha1$, Melatonin, Serotonin $5HT_{1A}$, Serotonin $5HT_{1B}$ receptors and site 2 of the Voltage-Gated Sodium Channel) and BIA 2-195 (5HT1A and 5HT1B receptors) are of little relevance due to their low levels *in vivo*. BIA 2-194 did not exhibit marked binding activity in the HTS assay.**

Secondary Pharmacology Studies:

Since BIA 2-093 and its metabolites are antagonists of the voltage gated sodium channels, the Sponsor hypothesized that this compound may exhibit analgesic activity. This hypothesis was tested using two different mouse models of pain sensation, the chronic constriction nerve injury test (CCI; Study 093-415) and the formalin late phase test (Study 093-421). Neither of these studies was conducted in accordance with GLP. BIA 2-093 exhibited an analgesic effect in both of these models.

In high throughput screening studies, BIA 2-093, BIA 2-194 and BIA 2-195 were shown to interact with the Adenosine A1, Dopamine D1, $GABA_A\alpha1$, Melatonin, Serotonin $5HT_{1A}$, Serotonin $5HT_{1B}$ receptors and site 2 of the Voltage-Gated Sodium Channel (Reviewer's Table 4.6). Specifically BIA 2-093 exhibited greater than or equal to 50% inhibition of the ligand binding to these receptors. BIA 2-194, the major human metabolite of BIA 2-093, did not exhibit marked binding inhibition (>50%) of any of the targets tested in the HTS. BIA 2-195 exhibited some binding activity at the Serotonin $5HT_{1A}$ and $5HT_{1B}$ receptor.

Targets	BIA 2-093	BIA 2-194	BIA 2-195
Adenosine, A1	97.7	32.3	-1.6
Dopamine, D1	49.4	6.9	7.3
GABAA, BDZ, α1	51.5	25.9	38.7
Melatonin, non-selective	55.3	17.4	30
Serotonin, $5HT_{1A}$	61.8	40.8	53.7
Serotonin, $5HT_{1B}$	58.7	45.5	75.9
Sodium Ion Channel, Site 2	65.9	34.5	16.9

Reviewer Table 4.6- HTS Assay for BIA2-093 Receptor Interaction. Data shown are % inhibition of binding of a specific ligand in the presence of 400 µM of the test article (Study 093-849). Significant interaction with the target is considered to be >50% inhibition.

In order to further understand the biological relevance of the targets that were identified in the HTS, the Sponsor determined the IC_{50} and K_i of the test article-receptor interactions. In Study 093-864, it was shown that the IC_{50} and K_i for the BIA 2-093 interaction with the adenosine A1 receptor were 22 µM and 11 µM, respectively. This interaction is 100 fold less potent than what is exhibited by the specific inhibitor of the adenosine receptor A1, 2-CADO. The IC_{50} and K_i were calculated to be greater than 1mM for the interaction of BIA 2-093 and both serotonin receptor isoforms. The IC_{50} and K_i of BIA 2-195 with the $5HT_{1A}$ receptor was shown in Study 09-865 to be 270 µM and 190 µM (Study #093-865), respectively. Due to the rapid and complete conversion of BIA 2-093 to its metabolites in humans, the marked *in vitro* interaction of BIA 2-093 with these receptors may be of little relevance.

4.3 Safety pharmacology

Reviewer's Summary:

- **BIA 2-093 and its metabolites did not alter hERG activity.**
- **BIA 2-093, BIA 2-005 and OXC caused QT/ QTc shortening *in vitro* and *in vivo*.**
- **BIA 2-093 caused long-lasting respiratory depression and an increase in enhanced pause, an index of airway obstruction, in mice and gastric hypomotility, sedation, hypothermia and decreased muscle tone in mice and rats.**
- **BIA 2-093 and its metabolites did not impair memory formation in the Passive Avoidance Test.**
- **BIA 2-093 exerted a species-dependent effect on diuresis/ natriuresis.**

In Vitro Studies:

The Sponsor conducted two types of *in vitro* safety pharmacology studies, hERG assays and the isolated cardiac Purkinje fiber test.

The Sponsor also tested the ability of BIA 2-093 and its metabolites to inhibit the function of the human ERG (hERG) potassium channel. Although, in general there is no clear relation between *in vitro* concentrations and plasma levels *in vivo*, the Sponsor chose the dose range in these studies to include a high dose which exceeded the expected plasma level (C_{max} at 1200 mg = ~ 23 µg/ml; Study 093-301) by >4 fold. There was, however, no clear characterization of a concentration-response curve as suggested in ICH Guidance S7B. The Sponsor does not state that the highest dose tested was limited by physiochemical parameters since it is stated that "no observations indicating instability of test item in bath solution was observed" suggesting that it may have been possible to test higher concentrations of the test articles in these assays. However, the overall impact of BIA 2-093 and its metabolites on the hERG current at the highest dose tested in each assay was trivial, suggesting that these test articles do not pose a risk for QT prolongation (Reviewer's Table 4.7).

Test Article	Study #	Highest Dose Tested (µM)	% Inhibition
BIA 2-093	093-853	337	19
BIA 2-194	093-855	393	8
BIA 2-195	093-856	393	0
BIA 2-005	093-854	393	15

Reviewer's Table 4.7: BIA 2-093 and Its Metabolites Did Not Markedly Inhibit the hERG Mediated Current In Vitro. HEK 293 cells stably expressing the hERG potassium channel were exposed to the test articles listed above in the table. The highest concentration tested in all assays was 100 µg/ml which is >4 fold higher than the human plasma concentration (C_{max}) expected in patients dosed with 1200 mg/day. BIA 2-005 is a racemic mixture of the S- and R-enantiomers of licarbazepine. All hERG studies were performed according to GLP and were subjected to QA audits.

In order to further examine if a risk for detrimental cardiovascular effects of the test articles existed, the Sponsor examined the impact of BIA 2-093, BIA 2-005 and OXC on action potential parameters in dog isolated cardiac Purkinje fibers (Study 093-857). The results of this study suggests a minimal risk for QT/ QTc prolongation since a delay in the repolarization of the Purkinje fibers was not observed in the presence of any of the test compounds. In the presence of the positive control dl-Sotalol, a marked lengthening of the repolarization was observed, consistent with its known ability to cause QT/ QTc prolongation. Although delay of the repolarization was not observed when Purkinje fibers were perfused with BIA 2-093, BIA 2-005 or OXC, a robust and dose-dependent shortening of the time to complete repolarization was observed (Table 5, Study 093-857) after fibers were perfused with these test articles. Specifically, the action potential duration (APD) measured at 60% and 90% of repolarization was shortened by as much as 48% (APD_{60}) and 28% (APD_{90}) respectively, when exposed to BIA 2-093. The racemic mixture of R- and S-licarbazepine (BIA 2-005) caused a similar shortening of APD. Finally, OXC exhibited a slightly more robust shortening of the parameters than was observed with BIA 2-093 (Table 4; Study 093-857). Overall, this study demonstrates that BIA 2-093, BIA 2-005 and OXC do not seem to pose a risk for prolonged QT/QTc. However, the robust decrease in the action potential duration suggests the potential for BIA 2-093, BIA 2-005 and OXC to cause QT/ QTc shortening. Although the exact toxicological relevance of QT/QTc shortening is not completely characterized, a recent publication suggests that QT/QTc shortening may be a risk factor for refractory or idiopathic ventricular fibrillation [1].

Table 4
Effect of Oxcarbazepine and Vehicle on Action Potential Parameters (0.5 Hz)

Action Potential Parameter	Baseline	Oxcarb 0.1 μg/ml	Oxcarb 1 μg/ml	Oxcarb 10 μg/ml	Oxcarb 100 μg/ml
RMP (mV)	-87.0 ± 1.3	-87.3 ± 0.9	-85.8 ± 0.9	-87.3 ± 1.4	-85.8 ± 1.9
UA (mV)	113.3 ± 2.5	112.0 ± 1.5	114.8 ± 1.4	111.0 ± 1.1	84.7 ± 4.1#
MRD (V/s)	534.8 ± 34.8	560.5 ± 43.7	572.3 ± 43.9	502.8 ± 59.7	168.0 ±42.5#
APD_{60} (ms)	227.0 ± 21.6	226.0 ± 27.2	219.3 ± 23.5	158.0 ± 11.6	83.7 ± 9.0#
APD_{90} (ms)	275.3 ± 19.5	275.0 ± 22.9	269.0 ± 19.2	209.0 ± 8.3	151.3 ±11.7#

Action Potential Parameter	Baseline	Vehicle 0.1%DMSO	Vehicle 0.1%DMSO	Vehicle 0.1%DMSO	Vehicle 0.1%DMSO
RMP (mV)	-86.9 ± 1.3	-86.6 ± 0.7	-86.1 ± 1.0	-86.7 ± 0.8	-87.0 ± 1.1
UA (mV)	113.3 ± 2.7	114.6 ± 2.2	115.6 ± 1.7	116.6 ± 1.5	114.0 ± 2.3
MRD (V/s)	518.6 ± 25.6	527.9 ± 36.0	536.3 ± 31.2	553.4 ± 35.8	539.4 ± 28.8
APD_{60} (ms)	199.7 ± 10.7	204.7 ± 11.8	206.1 ± 9.0	204.1 ± 10.1	214.9 ± 11.1
APD_{90} (ms)	253.7 ± 8.7	255.1 ± 8.2	258.1 ± 5.7	257.7 ± 8.5	268.4 ± 7.5

Values represent mean ± s.e. mean (n = 4 for oxcarbazepine and n = 7 for vehicle, except # where n = 3).
For abbreviations see text.

Table 5
BIA 2-093 and BIA 2-005:
Change from Baseline Action Potential Parameters (1 Hz)

Action Potential Parameter	BIA 2-093 0.1 μg/ml	BIA 2-093 1 μg/ml	BIA 2-093 10 μg/ml	BIA 2-093 100 μg/ml
RMP (mV)	0.8 ± 0.5	0.3 ± 0.8	-0.8 ± 0.8	1.0 ± 0.4
UA (mV)	-1.0 ± 1.1	0.0 ± 1.5	-3.0 ± 0.9	-24.3 ± 4.0**
MRD (V/s)	0.2 ± 4.4	-3.3 ± 4.0	-12.6 ± 2.6	-60.8 ± 7.8**
APD_{60} (ms)	-0.5 ± 1.2	-2.5 ± 0.5	-17.8 ± 2.5**	-45.8 ± 10.9**
APD_{90} (ms)	0.2 ± 0.7	-0.8 ± 0.3	-10.4 ± 1.7**	-25.6 ± 8.1**

Action Potential Parameter	BIA 2-005 0.1 μg/ml	BIA 2-005 1 μg/ml	BIA 2-005 10 μg/ml	BIA 2-005 100 μg/ml
RMP (mV)	0.3 ± 1.1	0.5 ± 0.5	1.8 ± 0.9	3.0 ± 1.8*
UA (mV)	6.5 ± 2.6	6.8 ± 1.9	3.3 ± 3.0	-10.5 ± 5.1
MRD (V/s)	10.9 ± 5.0	11.0 ± 8.8	6.3 ± 7.6	-49.3 ± 11.4**
APD_{60} (ms)	0.8 ± 1.3	-1.0 ± 1.5	-10.6 ± 2.6**	-45.2 ± 11.3**
APD_{90} (ms)	1.1 ± 1.5	0.3 ± 0.6	-5.0 ± 2.7*	-29.9 ± 8.1**

Values represent mean ± s.e. mean (n = 4 for each test substance),
* $P < 0.05$, ** $P < 0.01$.

Sponsor Table 5; Study 093-857: BIA 2-093 and BIA 2-005 Alter Electrophysiological Parameters Measured in Studies with Canine Purkinje Fibers.

In Vivo Studies:

Cardiovascular (Study #093-859 & 093-858):

Using an implantable telemetric device, the Sponsor examined the acute effect of BIA 2-093 on cardiovascular function in beagle dogs exposed to 40, 80, 210 mg/kg, delivered orally via gelatin capsule, in Study #093-858. Over the course of 24 hours, no dose related effects on diastolic, systolic or mean arterial blood pressure were observed in dogs exposed to BIA 2-093. However, an increase in heart rate (~44%, 130 bpm in HD vs. 90 bpm in controls), which coincided with the T_{max} (4.8 ± 3 hrs), was observed in the highest dose group (210 mg/kg; Figure 8, Study 093-858). Along with the increase in heart rate, a shortening of QT interval was observed (Figure 12, Study 093-858) up to 4 hours after dosing with 210 mg/kg. In order to determine if this shortening was due to the increased heart rate, the Sponsor applied two methods of QT correction, Fridericia's and van de Water's. Both methods of QT correction demonstrated that the QTc was shortened by 17-18 msec in the dogs receiving 210 mg/kg. This observation is consistent with the shortened action potential duration observed in the canine isolated Purkinje fiber study discussed earlier. The QTc shortening was associated with plasma levels of up to 2,307 ng/ml BIA 2-093, 25,700-35,214 ng/ml BIA 2-194 and 1,212-3,845 ng/ml oxcarbazepine.

In Study #093-859, the Sponsor further examined the effect of BIA 2-093 on cardiovascular function in the pentobarbitone-anesthetized male beagle dog. This study was performed according to GLP. Anesthetized dogs were dosed with 0 or 160 mg/kg BIA 2-093 (dissolved in 0.5% methylcellulose) intraduodenally. BIA 2-093 (160 mg/kg) did not markedly alter blood pressure, PR interval, QRS interval, QTc interval, left ventricular end-diastolic pressure, left ventricular systolic pressure, left ventricular $dP/dt.P^{-1}$, cardiac output, stroke volume, peripheral

resistance, mean femoral arterial blood flow and conductance. The results of this study are not that surprising considering that alteration of cardiovascular parameters were previously observed in Study 093-858 beginning at 210 mg/kg BIA 2-093. Therefore, the results of Study 093-859 do not necessarily contradict the previous finding that BIA 2-093, at a sufficiently high dose (210 mg/kg), can shorten the QTc interval *in vivo*.

Respiratory (Study #093-852 and 093-859):

Respiratory function was studied in pentobarbitone-anesthetized male beagle dogs dosed intraduodenally with 0 or 160 mg/kg BIA 2-093. No alteration of respiration rate, blood pH, pCO_2 or pO_2 was observed in the dogs dosed with BIA 2-093 suggesting that the test compound does not alter respiratory function in dogs at doses up to 160 mg/kg (Study 093-859). It is important to note that the highest dose tested in the cardiovascular study (210 mg/kg) was not tested in this study.

Whole body plethysmography was performed on mice dosed with BIA 2-093 by oral gavage to determine the effect of the test compound on respiratory function (Study 093-852). The effect of BIA 2-093 on respiratory function was compared to that of the positive control, theophylline. This study was conducted under GLP. A slight increase in the inspiratory time, defined as the time from the beginning of inspiration until the end of the same inspiration, was observed. This increase was short lived (<15 minutes) in the mice receiving 50 mg/kg BIA 2-093, but was also observed at 240 minutes after exposure in the mice receiving 100 and 200 mg/kg BIA 2-093. Expiratory time was also altered in mice dosed with BIA 2-093. Expiratory time was defined as the time between the end of inspiration and the beginning of the next inspiration. 200 mg/kg BIA 2-093 significantly increased the expiratory time to a maximum of 2.6-fold over baseline at 180 minutes post-administration. In addition, a decrease in the respiratory rate was observed in mice exposed to 200 mg/kg BIA 2-093. Mice exposed to 200 mg/kg BIA 2-093 also exhibited a significant increase in relaxation time and enhanced pause. Of these changes, the increase in enhanced pause is most meaningful since this is an index of airway obstruction. At the end of the testing period (240 minutes), the mice exposed to 50 or 100 mg/kg BIA 2-093 did not exhibit any behavioral side effects. However, loss of balance was observed in mice that received 200 mg/kg. Overall, the plethysmography studies suggest that BIA 2-093 at 200 mg/kg acts as a long-lasting respiratory depressant which can increase enhanced pause, a measure of airway obstruction in mice.

Gastrointestinal Motility (Study #093-863 and 093-862):

The effect of BIA 2-093 on gastric motility was measured in CD-1 mice and Sprague-Dawley rats using the charcoal gastric transit test. In mice exposed to 50, 100, 250 and 500 mg/kg BIA 2-093, a NOEL for decreased gastric motility (13-51%) could not be identified. In rats exposed to 15, 50 and 150 mg/kg BIA 2-093, it was determined that the NOEL for decreased gastric motility was 15 mg/kg. In summary, BIA 2-093 robustly decreases gastric motility in both rats and mice with a NOEL of 15 mg/kg.

Renal (093-861; 093-860):

In the diuresis/ natriuresis test in the CD1 mouse, the Sponsor demonstrated that exposure to single doses of BIA 2-093, by oral gavage, resulted in a dose-dependent decrease in urine production with a NOEL of 100 mg/kg. Specifically, forced urine production after an oral gavage bolus of saline was decreased by 62% in mice receiving a dose of 250 mg/kg BIA 2-093. Urine

production almost completely ceased (95%) in mice receiving 500 mg/kg BIA 2-093. Mice receiving 500 mg/kg of BIA 2-093 also exhibited marked sedation, lack of motor coordination, hypothermia and tremor. Furthermore, urinary sodium (29-54% at 250 and 500 mg/kg, respectively), potassium (38-66% at 250 and 500 mg/kg, respectively) and creatinine (20-60% at 250 and 500 mg/kg, respectively) concentrations were decreased in the same dose dependent fashion as urine volume.

The Sponsor also investigated the renal effects of BIA 2-093 (15, 50, 150 mg/kg) in the Sprague-Dawley rat. Unlike in the mouse, BIA 2-093 exhibited a diuretic effect (~2 fold increase in urine production) up to 6 hours post dose. The NOEL for diuresis was not determined since marked diuresis occurred at the lowest dose tested (15 mg/kg; 0-3 hrs sampling interval). In addition, an increase in urinary sodium (51-78%), potassium (290%) and chloride (44-73%) with a NOEL of 50 mg/kg was observed in rats between 3-6 hours after dosing when compared to controls. In summary, BIA 2-093 exerted a species dependent effect on diuresis/ natriuresis, with urine production decreased in mice but increased in rats.

Functional Observational Battery (093-408; 093-851; 093-850):

Using the Irwin Test and the Functional Observational Battery (FOB), the Sponsor examined the effect of BIA 2-093 on the behavior and basic functioning of both mice and rats. The studies were conducted by GLP. Male CD-1 mice were exposed to a single oral dose of 50, 100, 250 or 500 mg/kg BIA 2-093 (Study 093-850). The NOAEL in this study was 50 mg/kg. The next highest dose (100 mg/kg) resulted in slight hypothermia during the first hour of observation. The symptoms observed increased in severity as the dose was increased to 250 and 500 mg/kg. Specifically, at 250 mg/kg moderate to marked sedation and decreased muscle tone were observed in all 6 mice for up to three hours post dosing. At 500 mg/kg, convulsions and Straub tail were observed in 1/6 mice, but sedation was observed in all 6 mice. In addition, rolling gait in 3/6 mice, decreased muscle tone in 6/6 mice and loss of traction in 3/6 mice were observed. Hypothermia also occurred in mice dosed with 250 and 500 mg/kg and persisted for up to three hours after dosing. In summary, BIA 2-093 exhibited a dose dependent increase in sedation, hypothermia and decreased muscle tone with a NOEL of 50 mg/kg in CD-1 mice.

Sprague-Dawley rats (Study 093-851) were administered a single 15, 50 or 150 mg/kg oral dose of BIA 2-093 and then examined in the Irwin Test. Chlorpromazine (20 mg/kg) , a CNS depressant, was used as the positive control. The NOAEL was determined to be 50 mg/kg with the highest dose of 150 mg/kg causing symptoms consistent with CNS depression, such as decreased grip strength and locomotor activity and increased apathy. Consistent with the observations in BIA 2-093 exposed CD-1 mice and Sprague Dawley rats, Wistar rats exposed to BIA 2-093 (Study 093-408) exhibited symptoms of CNS depression such as altered righting reflex, decreased muscle tone, altered Rotarod performance, decreased open field activity, decreased time spent rearing and increased latency in the two-way active avoidance trial. The NOAEL observed in this study was 100 mg/kg. Mice dosed with up to 100 mg/kg BIA 2-093, BIA 2-194, BIA 2-195 (p.o.) did not exhibit impaired memory formation in the Passive Avoidance task (Study 093-873).

Safety Pharmacology Summary:

The Sponsor provides evidence in the safety pharmacology studies to suggest that BIA 2-093 may alter the normal functioning of several organ systems (Reviewer's Table 4.8). In particular, a robust inhibition of gastrointestinal motility occurred in both mice and rats exposed to BIA 2-

093. However, measurement of blood levels of the parent compound and its metabolites was not performed which makes it difficult to calculate safety margins for this effect in humans. Another possible safety pharmacology issue of concern is the potential of BIA 2-093 to cause a shortening of the QT/QTc interval. Both *in vitro* evidence from isolated Purkinje fibers and *in vivo* canine studies demonstrate that BIA 2-093 is capable of shortening the QT/QTc interval, an alteration associated with idiopathic ventricular fibrillation [1]. The diuretic and oliguretic effect of BIA 2-093 in rats and mice, respectively, may be related to the difference in *in vivo* metabolism that exists between these species. Finally, BIA 2-093 causes central nervous system depression in both rats and mice.

Adverse Effect (Organ System)	**Rat NOEL (mg/kg)**	**Mouse NOEL (mg/kg)**	**Dog NOEL (mg/kg)**
QTc shortening (Cardiovascular)	---	---	160
Depression/ Obstruction (Respiratory)	---	100	160 **
Decreased motility (Gastrointestinal)	15	< 50 *	---
Altered Urine Production (Renal)	15*	100	---
Depressant/ myorelaxant (Nervous System)	50	50	---

Reviewer's Table 4.8: Summary Table of Safety Pharmacology Studies with BIA 2-093.= No NOEL determined. **= BIA 2-093 was delivered intraduodenally. Dosing in all other studies was via the oral route.*

5. Pharmacokinetics

Reviewer's Summary:

- **The methods developed to measure BIA 2-093 and its metabolites in biological media are adequate.**
- **The half-life of BIA 2-005 differs among animals used in non-clinical studies (rats = 4.5-18 hrs; mice= 1.1-8.5 hrs; dogs = 1.1-4.8 hrs).**
- **BIA 2-093 is transported into cells via passive diffusion.**
- **BIA 2-093, BIA 2-194 and BIA 2-195 all possess the ability to cross the BBB. However, BIA 2-194 and BIA 2-195 are subject to efflux from the brain by p-glycoprotein and multidrug resistance protein.**
- **BIA 2-093 and its metabolites distribute mainly to organs of biotransformation (i.e. liver), elimination (i.e., kidney) and secretion (i.e. pancreas, preputial gland).**
- **The conversion of BIA 2-093 to BIA 2-194 is independent of CYP450 activity.**
- **Urine is the major route of elimination in mice and dogs exposed to BIA 2-093. Feces was the main route in rats.**
- **BIA 2-093 and/ or its metabolites are transferred to suckling pups via milk.**
- **AEDs do not prevent the *in vitro* metabolism of BIA 2-093 to BIA 2-194.**
- **Phenytoin and tolbutamide decrease the binding of BIA 2-194 to human plasma proteins, *in vitro*.**
- **No conclusive evidence that BIA 2-194 will not alter the plasma protein binding of warfarin, diazepam, digoxin, phenytoin and tolbutamide *in vitro*.**
- **BIA 2-194 dose-dependently inhibits CYP2C9 *in vitro* but induces CYP 2A6, 2C9, 2E1, 2C19 and CYP 3A4 *in vivo*. BIA 2-194, BIA 2-195 and OXC inhibit CYP2C19 *in vitro*.**

Analytical Methods and Validation Reports:

In Study Report #'s 093-500 to 093-513, the Sponsor described the validation of the HPLC method used for measuring BIA 2-093 and its metabolites in the vehicle and biological media. These studies also investigated the stability of this material in the dosing vehicle and biological media. Stability of BIA 2-093 in 0.5% hydroxypropylmethylcellulose (HPMC) was assessed over the course of 30 days at both room temperature and between 2-8^{o}C. Dosing solutions of 4 or 200 mg/ml exhibited a stability of between 94.0 to 108% when protected from light. No interfering peaks were observed in reference standards for BIA 2-194 (Study 093-503) and BIA 2-195 (Study 093-504). The % Relative Standard Deviation (RSD) for system precision of the assay developed by the Sponsor to measure BIA 2-093 in the dosing vehicle was calculated to be 0.1%. The % RSD for repeatability of the assay was 0.8%. Overall, study 093-513 demonstrated that the method developed for measuring BIA 2-093 in the dosing vehicle is acceptable. A similar study to determine if the assay developed to measure BIA 2-194 in the dosing vehicle was acceptable was performed by the Sponsor (Study 093-506). In this study, it was determined that the system precision was calculated to be 0.1% and the repeatability was 0.8%. Also, no interfering peaks were observed for the peak corresponding to BIA 2-194. The method to measure BIA 2-195 in the dosing vehicle was determined to have a precision of 0.5% and a repeatability of 0.5% with no interfering peaks (Study 093-507). Stability of BIA 2-195 in the dosing vehicle was determined to be between 97.6% and 100.8% at ambient temperature and 4^{o}C for two days.

Studies 093-508 and 093-509 describe the development and validation of an LC-MS/MS method for the measurement of BIA 2-093, BIA 2-194, BIA 2-195 and oxcarbazepine in dog, rat and mouse plasma. BIA 2-093, BIA 2-005 and OXC were shown to be unstable in rat plasma stored at room temperature for 24 hours. (b) (4) samples were collected into tubes containing dichlorvos ((b) (4) placed on ice, centrifuged and stored frozen. BIA 2-093, BIA 2-005 and OXC are stable up to 38.5 h at 8 ± 5°C after extraction. BIA 2-093 and BIA 2-005, but not OXC, are stable in dog and mouse plasma for up to 24 hours at room temperature. (b) (4) the same approach described above for rat plasma was followed for mouse and dog plasma samples used for TK analysis. BIA 2-194, BIA 2-195 and OXC are stable up to 18 hours in mouse plasma at room temperature. BIA 2-194 and BIA 2-195, but not OXC, are stable after multiple freeze-thaw cycles (>3 cycles) in dog, mouse and rat plasma. Peaks obtained from LC-MS/MS analysis were well resolved for all analytes. Since BIA 2-194 and BIA 2-195 are stereoisomers, the Sponsor developed a chiral LC-MS/MS method to distinguish between these two analytes in rat and dog plasma (Study # 093-510 and 093-513). In study 093-510, it was determined that OXC along with BIA 2-194 and BIA 2-195 could be measured using the developed method. The method was determined to be linear up to 10,000 ng/ml for BIA 2-194 and 2-195. The peaks for BIA 2-194 (RT=3.14 min) and BIA 2-195 (RT=2.47 min) were well resolved, suggesting acceptable specificity of the method. Confirming the previous observation, BIA 2-093 was shown to be unstable in rat plasma unless enzyme activity was reduced via multiple freeze thaw cycles of the plasma before BIA 2-093 was spiked into the matrix. Aside from slightly different retention times, similar results were obtained using a matrix of dog plasma (Study 093-513) and rabbit plasma (Study 093-512). Validation of an HPLC-UV method for quantification of BIA 2-093, OXC and the 10,11-Dihydro metabolite of BIA 2-093 was also performed by the Sponsor (Study # 093-511).

Overall, the LC-MS/MS and HPLC-UV methods developed to measure BIA 2-093 and its metabolites represent an acceptable methodology for determining the toxicokinetic and pharmacokinetic parameters of BIA 2-093 and its metabolites in nonclinical studies.

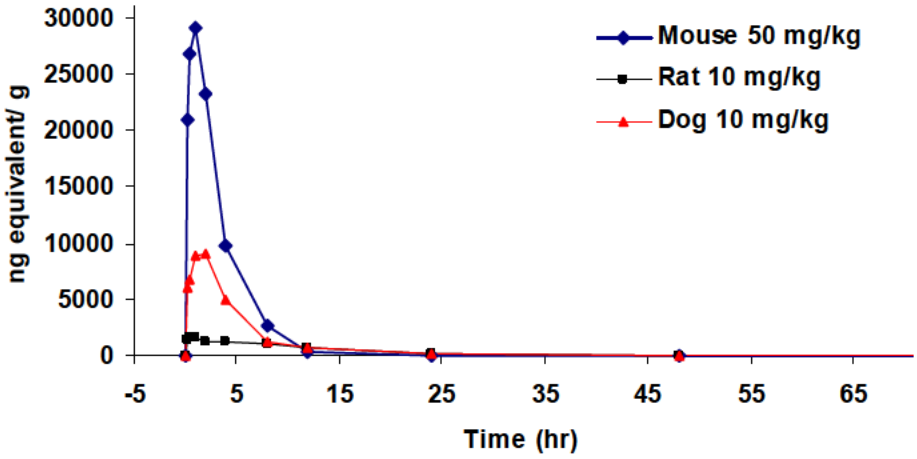
PK/ADME:

The Sponsor has extensively studied the *in vivo* and *in vitro* PK/ADME of BIA 2-093. In general, the parent compound was not detected in plasma from animals exposed chronically to BIA 2-093 due to the fact that BIA 2-093 is extensively metabolized to the monohydroxy metabolite and OXC. Early in the development of BIA 2-093, the Sponsor measured the concentration of the metabolite in a manner irrespective of its isomeric orientation and referred to this stereoisomeric mixture as BIA 2-005. Overall, the half-life of BIA 2-005 varies between non-clinical animal models (rats= 4.5-18 hrs; mice = 1.1-8.5 hrs, dogs 1.1-4.8 hrs). The half-life of OXC, a minor metabolite of BIA 2-093 in mice, dogs and humans but a major metabolite in rats, also varied among species but generally had a half-life similar to that for BIA 2-005 (rats= 3.3-11.6 hrs; 1.8-4.6 hrs, 0.7-6.3 hrs). In the toxicokinetic analysis performed in mice and dogs, a relatively linear, dose-proportional increase in BIA 2-005 plasma concentrations (measured as $AUC_{0\text{-}24hr}$) was observed. Furthermore, male mice consistently exhibited greater plasma BIA 2-005 concentrations than females while plasma concentrations of BIA 2-005 were similar in male and female dogs. Plasma concentrations of

BIA 2-005 in rats were generally lower than the concentration of OXC. Finally, the C_{max} of BIA 2-093 metabolites followed the same pattern observed for $AUC_{0\text{-}24hrs}$.

Absorption:

Although the magnitude of the plasma levels differs between species, the time to peak plasma concentration (T_{max}) of [^{14}C]-BIA 2-093 occurs at approximately one hour in rats, mice and dogs (Study 093-514, 093-516, 093-518; Reviewer's Figure 5.1). Other PK parameters, such as half life, were not calculated in these rat, mouse or dog studies.



Reviewer's Figure 5.1: Plasma Levels of [^{14}C]-BIA 2-093 Peak at One Hour in Mice, Rats and Dog After a Single Oral Dose. Time is elapsed time since single oral dose. Data are from Studies 093-514, 093-516, 093-518.

In order to understand how BIA 2-093 and its metabolites are absorbed and transported at the cellular level, the Sponsor conducted an *in vitro* study in Caco2 cells (Study 093-519). Due to the fact that verapamil, MK 571 and DNP did not disrupt the transport of BIA 2-093 in this study, it is suggested by the Sponsor that BIA 2-093 is transported by passive diffusion. This interpretation is consistent with the physiochemical properties of BIA 2-093 that suggest it is lipophilic and nonionizable under physiological conditions. Supporting these results, the Sponsor demonstrated that the apparent permeability (P_{app}) of BIA 2-093 in Caco2 cells was 2.3 $x10^{-5}$ to 4.2 $x10^{-5}$ cm/s (Study 093-535); values consistent with the characteristic of a highly permeable compound ($P_{app} > 1x10^{-5}$ cm/s). Passive transport of the hydroxylated metabolites of BIA 2-093, BIA 2-194 and 2-195, is less robust that the parent compound in Caco2 cells.

Distribution:

The sponsor employed several techniques to characterize the distribution of BIA 2-093. Specifically, whole body phosphor imaging (WBPI), *in vitro* plasma protein binding assays, cerebral spinal fluid analysis and *in vivo* microdialysis were performed.

In the WBPI studies (093-520 and 093-522), mice and rats were exposed to a single oral dose of [^{14}C]-BIA 2-093 (50 mg/kg and 10 mg/kg, respectively) before being euthanized at time points ranging from 0.5 and 48 hours post dose. In mice exposed to 50 mg/kg [^{14}C]-BIA 2-093, the radioactivity was well distributed throughout the body within one hour after

dosing with the gall bladder, stomach, urine, and small intestine contributing markedly to the overall signal (Study 093-520). The same overall pattern of distribution persisted with the exception of a major increase in the amount of radioactivity detected in the large intestine and cecum. At 12 hours, radioactivity was only detected in the cecum, bile, kidney, large and small intestines, stomach and urine. At 48 hours, small but detectible amounts of radioactivity were only detected in the small and large intestines and cecum. When normalized to blood levels, only the kidneys, liver, pancreas, salivary gland, thyroid, and adrenal medulla exhibited a tissue to blood ratio of >1 at one hour after dosing. At four hours, only the liver and kidneys exhibited a ratio of >1. These data suggest that the test article does not specifically accumulate in any organ, is cleared mainly via the bile and urine and may distribute to secretory glands such as thyroid and pancreas. Finally, BIA 2-093 or its metabolites seem to cross the blood brain barrier since a radioactive signal was observed in the brain for up to 4 hours after exposure in mice (brain: blood ratio of 0.33).

In rats, a similar profile of distribution was observed with a few notable differences. As was observed in mice, radioactivity was well dispersed throughout the animal one hour after dosing with 10 mg/kg [^{14}C]-BIA 2-093. At this time point, urine, stomach, small intestine and the preputial gland accounted for areas with the greatest distribution. The pattern persisted at four hours with the radioactivity levels increasing markedly in the cecum and large intestine compared to the amounts detected at one hour after dosing. Unlike what was observed in the mouse, marked levels of radioactivity could be detected in all organs at 12 hours after dosing except for the arterial wall, bone marrow, eye humor, and white fat. Furthermore, at 48 hours after dosing, small but quantifiable levels of radioactivity were still detected in the cecum, kidney, large intestine, liver, nasal mucosa, small intestine, stomach, thyroid and urine. Interestingly, at this time point, the preputial gland retained 16 % of the maximal amount of radioactivity observed in this organ. Most other organs and glands, with the exception of the small and large intestines and cecum, retained less than 5-10% of the maximal radioactivity quantified at that specific organ or gland. This suggests that clearance of BIA 2-093 and its metabolites from the preputial gland in rats is protracted. When normalized to blood levels, it is clear that distribution of [^{14}C]-BIA 2-093 to the adrenal glands, arterial walls, cardiac muscle, epididymis, Harderian gland, kidney, liver, lung, pancreas, preputial gland, and the thyroid occurs within one hour after dosing. Except for the arterial wall, this accumulation was observed up to 12 hours, the latest time point for which data of this nature was provided by the Sponsor. The accumulation of radioactivity was quite remarkable in the preputial gland which achieved tissue to blood ratios of 10, 35 and 87 at one, four and twelve hours post dosing, respectively. Finally, although radioactivity was observed in the brain up to 12 hours after dosing, the tissue to blood ratios were consistently less than 1 at all time point suggesting that BIA 2-093 and its metabolites do not accumulate in the brain. BIA 2-093 was also shown to be able to cross the mouse placenta into the fetus (Study 093-536).

Overall, these rodent distribution studies suggest that there is some entry but little accumulation of BIA 2-093 and its metabolites in the brain. Also, it is evident that BIA 2-093 and its metabolites distribute mainly to organs of biotransformation (i.e. liver), elimination (i.e., kidney) and secretion (i.e. pancreas, preputial gland).

Since BIA 2-093 was designed to be a prodrug, it was important for the Sponsor to further characterize the ability of its metabolites to distribute to the brain. The ability of the metabolites of BIA 2-093 to cross the blood brain barrier was studied in both mice and

cynomolgus monkeys. In NMRI mice (Study 093-521), the Sponsor used *in vivo* microdialysis to investigate the involvement of p-glycoprotein (p-gly) and MRP on the efflux of BIA 2-194 and BIA 2-195 across the blood brain barrier (BBB). BIA 2-194 or BIA 2-195 were administered by intraperitoneal injection and the amount of test substance detected by microdialysis probes implanted into the brain was determined in the presence or absence of an intracerebral injection of probenecid or verapamil, known inhibitors of the multidrug transporters in the BBB. Verapamil, a p-gly inhibitor, significantly increased the $AUC_{0\text{-}240min}$ of BIA 2-194 and BIA 2-195 in the brain by 67% and 73%, suggesting that these compounds are substrates for p-gly mediated brain efflux. Probenecid, an inhibitor of MRP, increased $AUC_{0\text{-}240min}$ of BIA 2-194 and BIA 2-195 in the brain by 39% and 41% suggesting that these compounds are also substrates for MRP. These studies show that BIA 2-194 and 2-195 enter the brain and that the brain levels of these molecules are, in part, controlled by BBB p-gly and MRP activity. The cerebrospinal fluid (CSF) and plasma of cynomolgous monkeys receiving a single dose of BIA 2-093 or OXC was monitored to characterize the ability of these compounds to enter the central nervous system (Study 093-523). The main metabolite detected in the CSF after a single i.v. injection of BIA 2-093 was BIA 2-194 (99.2%). BIA 2-195 was not detected in the CSF or plasma of monkeys receiving BIA 2-093. Very low levels of OXC (0.2%) were detected in the CSF and plasma of monkeys receiving BIA 2-093. The results of this study suggest that the main active metabolite of BIA 2-093 in the CSF of monkeys is BIA 2-194.

Finally, the interaction of BIA 2-093 and BIA 2-194 with plasma proteins and blood cells was also characterized by the Sponsor (Study 093-524 and 093-525). This attribute can play an important role in determining the free fraction available to distribute to and interact with biological targets. In Study 093-524, rat, dog and human plasma were incubated with [^{14}C]-BIA 2-093 and the free fraction (concentration in ultrafiltrate/ concentration in unfiltered plasma) was determined by ultrafiltration. It was determined that there was a species dependence regarding the extent of plasma protein binding for BIA 2-093. Humans exhibited the greatest amount of *in vitro* plasma protein binding with 60.7% bound. Dogs exhibited 40.5% bound and rats exhibited the lowest amount of binding to plasma proteins with 28.2% bound. Binding of radiolabelled BIA 2-093 to blood cells was in the range of 33-37% for all 3 species tested. Although radiolabelled BIA 2-093 was incubated with plasma and blood cells in these assays, it is impossible to determine which metabolites of BIA 2-093 contribute to the binding. Therefore, the Sponsor repeated this assay in the presence of radiolabelled BIA 2-194 (Study 093-525). In this study, it was demonstrated that for BIA 2-194, the binding to plasma proteins was similar between species with 25.4% bound in rats and 30.1% bound in humans. The binding to blood cells in humans (46.2%) was similar to the extent of blood cell binding in rats (43.1%). Binding in mice (Study 093-537), exhibited a low degree of plasma binding (~24%) and a moderate level of binding to blood cells (49.5%). Overall, the low level of plasma protein and blood cell binding suggest that interaction of BIA 2-093 and its metabolites with elements of blood is of little clinical significance.

Metabolism:

As BIA 2-093 is a pro-drug, it is important to understand its metabolism to BIA 2-194 and other potentially bioactive metabolites. The Sponsor characterized the *in vitro* metabolism by incubating radiolabelled BIA 2-093 with rat, dog, primate and human liver microsomes (Study 093-526). In microsomes from all species tested, the main metabolite was BIA 2-194

in the presence and absence of cofactors for CYP 450 metabolism. This result suggests that the conversion of BIA 2-093 to BIA 2-194 is mainly independent of CYP450 activity. OXC was also detected as a minor metabolite in each species. This *in vitro* finding is inconsistent with the metabolism of BIA 2-093 in intact rats where OXC is the major circulating metabolite. Furthermore, since the Sponsor did not measure the metabolism of BIA 2-093 in the rat liver S9 fraction, the metabolite profile in the *in vitro* genotoxicity assays is unknown. Other minor unidentified metabolites (Reviewer's Table 5.2) were observed in assays from all species except monkey in the presence, but not in the absence, of an NADPH generating system. This suggests that the formation of these minor, unidentified metabolites is dependant on CYP450 activity. The proposed metabolic schematic provided by the Sponsor is presented below.

Metabolites Produced in the Presence of NADPH Generating System

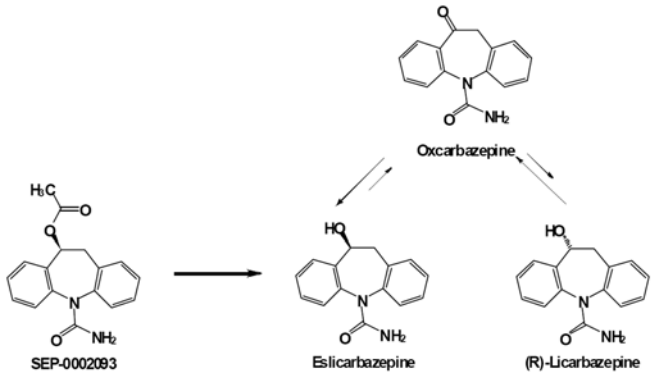
Animal	1.6 min	4.5 min	11.1 min	BIA 2-194 (13.3 min)	OXC (18.3 min)	BIA 2-093 remaining
Human	1.35	0	0.958	93.2	2.6	1.9
Rat	6.1	1.9	4.4	75.7	4.3	7.6
Dog	2.1	4.2	2.5	90.1	1.1	0
Monkey	5.8	4.66	2.8	78.2	4.6	3.9

Metabolites Produced in the Absence of NADPH Generating System

Animal	1.6 min	4.5 min	11.1 min	BIA 2-194 (13.3 min)	OXC (18.3 min)	BIA 2-093 remaining
Human	0	0	0	98.8	1.2	0
Rat	0	0	0	98.8	1.2	0
Dog	0	0	0	100	0	0
Monkey	0	0.7	0.9	94.1	3.5	0.7

Reviewer's Table 5.2: Hydrolysis of BIA 2-093 to BIA 2-194 is Independent of CYP450 Activity. Column headings are the retention time (RT) of the metabolite under the HPLC conditions employed by the Sponsor. Data are % of total peak area detected. The identity of the metabolites exhibiting RTs of 1.8, 4.5 and 11.1 min are unknown. OXC=oxcarbazepine. The results presented above represent the final time point (10 minute incubation) from Study 093-526. Metabolites formed in the absence of the NADPH generating system are likely produced via a CYP450 independent mechanism.

Metabolism of BIA 2-093 (Eslicarbazepine Acetate)

	% of Circulating Species that Correspond to Parent and Metabolite Following Oral Administration of SEP-0002093*			
				
	SEP-0002093	Oxcarbazepine	Eslicarbazepine	(R)-Licarbazepine
Human	-	1%	95%	4%
Dog	8%	4%	88%	-
Rabbit	-	7%	93%	<1%
Rat	-	86%	13%	1%
Mouse	-	25%	74%	<1%

BIA 2-093 OXC BIA 2-194 BIA 2-195

Elimination:

In Studies 093-514, 093-516, and 093-518, the Sponsor determined the major routes of excretion in mice, rats and dogs exposed to intravenous doses of radiolabelled BIA 2-093 (Reviewer's Table 5.3). Urine was determined to be the major route of elimination in mice and dogs exposed to BIA 2-093. Feces were the major route of elimination in rats exposed to BIA 2-093.

Species	Urine (%)	Feces (%)
Mice	53.7	32.9
Rat	26.2	59.1
Dog	64.4	22.8

Reviewer's Table 5.3: *Major Routes of Elimination in BIA 2-093 Exposed Animals. The data provided is the mean percentage of administered radioactivity detected in the urine or feces. Data are from Studies 093-514, 093-516, 093-518.*

The Sponsor also investigated if BIA 2-093 and its metabolites are secreted via milk in the mouse (Study 093-527). Dams were given a single oral dose of 150 mg/kg of radiolabelled BIA 2-093 at PND 4 and the pups were allowed to suckle. The results of this study suggests that BIA 2-093 and/ or its metabolites pass into the milk of lactating female mice and can be transferred to the suckling pups (BIA 2-093 detected in pups (ng equiv/g) at 1hr= 239, 4 hrs =3221, 12 hrs= 3566, 24 hrs= 2170). Plasma and milk levels of BIA 2-093 or its metabolites were not measured in this study.

Pharmacokinetic Drug Interactions (PDI; nonclinical):

As BIA 2-093 is intended for use in patients with epilepsy, it is important to understand its ability to cause PDI. Therefore, the Sponsor examined if other anti-epileptic drugs (AED) alter the metabolism of BIA 2-093 (Study 093-528), if the plasma binding of BIA 2-194 can be altered by other pharmaceutical compounds (093-529), and if BIA 2-093 could alter the

activity of specific CYP450 isoforms (Study #'s 093-530, 093-531,093-532, 093-533, 093-534; 093-538).

The Sponsor demonstrated that acetazolamide, clobazam, clonazepam, gabapentin, lamotrigine, phenobarbital, phenytoin, primidone, and sodium valproate, when co-incubated with human liver microsomes and BIA 2-093, did not prevent the conversion of BIA 2-093 to BIA 2-194 (Study 093-528). Therefore, a PK drug interaction between AEDs and BIA 2-093, with respect to the production of BIA 2-194, is not expected *in vivo*.

Since BIA 2-194 is the major circulating metabolite of BIA 2-093, it is important to know if there is any potential for a PDI based on plasma protein binding. Therefore, the Sponsor examined the effect of warfarin, diazepam, digoxin, phenytoin and tolbutamide on the plasma protein binding of BIA 2-194 (Study 093-529). In addition, the Sponsor examined if BIA 2-194 exerted an effect on the plasma protein binding of any of the aforementioned compounds. When co-incubated with 5 µg/ml warfarin, diazepam or digoxin, concentrations that exceed the peak clinical plasma concentration by 5, 16 and 5000 fold [2], respectively, the binding characteristics of BIA 2-194 in human plasma were not altered. However, plasma protein binding of BIA 2-194 decreased from 37.2% to 28% in the presence of 10 µg/ml phenytoin and 26% in the presence of 20 µg/ml tolbutamide. Since the concentrations of phenytoin and tolbutamide used in this assay are lower than the peak clinical plasma concentration by two fold and 5 fold respectively [2], it is possible that clinically relevant concentrations of these drugs may result in further displacement of BIA 2-194 from plasma proteins. When the converse experiment was performed it was demonstrated that 10 µg/ml BIA 2-194 did not result in a change in the plasma protein binding activity of warfarin, diazepam, digoxin, phenytoin and tolbutamide. A caveat to this experiment is that the Sponsor did demonstrate in Study 093-301 that maximum plasma concentrations in patients treated in a repeat dose Phase 3 study may reach as high as 22.9 µg/ml BIA 2-194. Therefore, the concentration of BIA 2-194 used in Study 093-529 does not represent a peak clinical plasma concentration. As a consequence, the *in vitro* study does not provide conclusive evidence that BIA 2-194 will not alter the plasma protein binding of warfarin, diazepam, digoxin, phenytoin and tolbutamide in patients.

Finally, the Sponsor investigated if BIA 2-093 or its metabolites can induce or inhibit CYP450 activity. Using human hepatocytes, the Sponsor demonstrated in Study 093-533 that BIA 2-194 is not an inducer of CYP1A, CYP2C19 or CYP3A4 activity. The Sponsor further demonstrated in Study 093-534 that BIA 2-195 and OXC also do not induce CYP3A4. However, *in vivo* studies contradict these *in vitro* studies in human hepatocytes. Specifically, in mice, CYP 2A6, 2C9, 2C19, 2E1 and 3A4 activity was induced after 5 days of exposure to BIA 2-093 (Study 093-538). In a screen of CYP450 and certain Phase 2 enzyme activity in human microsomes (Study 093-530), the Sponsor provides presumptive evidence that CYP2C9 activity may be inhibited by 10%, 19% and 38% at 10, 50 and 100 µg/ml BIA 2-194, respectively. Finally, it was also shown in Studies 093-531 and 093-532 that BIA 2-194, BIA 2-195 and OXC dose dependently inhibit the activity of CYP2C19.

6. General Toxicology

Reviewer's Summary:

- **BIA 2-194 is the major circulating metabolite in human, mouse, and dog. Therefore, both the dog and the mouse are relevant test species since their metabolism of BIA 2-093 is similar to the human. The rat, on the other hand, is not a relevant test species for human safety assessment of BIA 2-093 due to its robust metabolism of BIA 2-093 to OXC, a minor metabolite in humans.**
- **The Sponsor has satisfied the repeat study duration recommendation of ICH M3(R2) for drugs intended for chronic administration in humans with a 6 month rodent and 9 month non-rodent study.**
- **Toxicities of potential relevance that were detected in the general toxicity studies are: 1) symptoms of seizure-like activity observed in mice and dogs; 2) consistently elevated APTT and cholesterol in dogs and 3) oliguresis in mice and decreased urinary electrolytes in dogs.**

6.1 Single-dose toxicity

Reviewer's Summary:

- **The NOAEL for acute exposure of mice to BIA 2-093 is 150 mg/kg (oral) and < 100 mg/kg (IV).**
- **The NOAEL for acute exposure of mice to (b) (4) is 150 mg/kg (oral) and 50 mg/kg (IV).**
- **The NOAEL for acute exposure of rats to BIA 2-093 is 300 mg/kg (oral) and < 50 mg/kg (IV).**
- **The NOAEL for acute exposure of rats to (b) (4) is 150 mg/kg (oral) and < 25 mg/kg (IV).**

The Sponsor studied the acute oral and intravenous toxicity of BIA 2-093 and (b) (4), in both mice and rats.

CD-1 mice in Study 093-801 (b) (4) 7/24/1997) were dosed with a single dose of 150, 300, 500 mg/kg BIA 2-093 (lot # PC96.03.22; purity= 100.7%) by oral gavage and euthanized 14 days later. Male and female mice exposed to 150 and 300 mg/kg did not exhibit clinical signs. Mice exposed to 500 mg/kg exhibited abnormal gait (4/5 F; 4/5 M), piloerection (0/5 F; 3/5 M), subdued behavior (4/5 F; 5/5 M). Clinical signs subsided by the end of the first day of observations. One female mouse exposed to 500 mg/kg was euthanized *in extremis* on day one. Total BW gain was decreased by 44-53% in males exposed to 300 and 500 mg/kg when compared to mice exposed to 150 mg/kg. BW gain was the same in all female dose groups. In summary, the NOEL for clinical signs such as abnormal gait, subdued nature and piloerection in mice is 300 mg/kg BIA 2-093 and the NOAEL for this study was 150 mg/kg.

CD-1 mice in Study 093-802 (b) (4) 7/24/1997) were dosed with a single intravenous dose of 100 mg/kg BIA 2-093 (lot # PC96.03.22; purity= 100.7%) and euthanized 14 days later. Death was observed in mice exposed to an IV bolus of 100 mg/kg BIA 2-093 (0/5 M; 1/5 F). On the first day of observation all males and females were prostrate and exhibited gasping, labored breathing. In summary, 100 mg/kg is an IV lethal dose of BIA 2-093 in mice. A NOAEL could not be determined since only one dose was tested in this study but is estimated to be < 100 mg/kg.

CD-1 CRL: CD-1 (CR) mice in Study 093-841 [redacted] 11/10/2004) were dosed by oral gavage with a single dose of 150, 500 or 1000 mg/kg [redacted] (lot# PC040831; Purity 99.9%) and euthanized 14 days later. Mice exposed to 1000 mg/kg were euthanized *in extremis* on Day 1. No mortality was observed in any other dose group. No clinical signs were observed at 150 mg/kg. Mice exposed to 500 mg/kg exhibited hunched posture, subdued behavior, piloerection and staggering until 4 hours post dose. Mice exposed to 1000 mg/kg exhibited convulsions, prostration, labored breathing and were cold to the touch. No abnormalities were observed in the gross pathology examination when animals were euthanized 14 days after dosing. The oral NOAEL for this study was considered to be 150 mg/kg [redacted] based on clinical signs at higher doses.

CD-1 CRL: CD-1 (ICR) mice in Study 093-842 [redacted] 11/10/2004) were dosed with a single intravenous dose of 50 of 100 mg/kg of [redacted] (lot# PC040831; Purity = 99.9%) and euthanized 14 days later. No clinical signs were observed in mice exposed to 50 mg/kg. Dark tails were noticed in 3/3 males and 1/3 females in the 100 mg/kg dose group. This finding was still present 14 days after injection in 1/3 males and in 1/3 females. No abnormalities were observed in females exposed to 50 mg/kg and in all males. The IV NOAEL for this study was determined to be 50 mg/kg [redacted]. Injection site abnormalities (black tail) were observed in mice receiving an IV injection of 100 mg/kg [redacted].

Sprague-Dawley rats in Study 093-803 [redacted] 7/24/1997) were dosed by oral gavage with a single dose of 150, 300 or 500 mg/kg BIA 2-093 and euthanized 14 days later. Male and female rats exposed to 150 and 300 mg/kg did not exhibit any abnormal clinical signs. On the day of dosing, 5/5 males and 5/5 females exposed to 500 mg/kg exhibited abnormal gait, piloerection and subdued behavior. These signs subsided within 3 hours after dosing and were not observed during the 14 day observation period. BW gain was decreased by 29% in males exposed to 500 mg/kg when compared to males exposed to 150 mg/kg. No abnormal weight gain was observed in females. Food consumption was not altered in any dose group. No abnormalities were observed in any dose group except for one male exposed to 300 mg/kg which exhibited small testes. The NOAEL for this study was determined to be 300 mg/kg, based on clinical signs and decreased BW gain at the HD.

Sprague-Dawley rats in Study 093-804 [redacted] 7/24/1997) were dosed with a single intravenous dose of 50 or 100 mg/kg BIA 2-093 and euthanized 14 days later. All rats (5/5 M and 5/5 F) exposed to 100 mg/kg were either euthanized *in extremis* or found dead on the day of dosing. Males (2/5) and females (2/5) exposed to 50 mg/kg died on the day of dosing. All rats exposed to BIA 2-093 exhibited gasping, dull eyes and were prostrate after dosing. All males (5/5) and 3/5 females exposed to 100 mg/kg BIA 2-093 exhibited distended bladders filled with red fluid. No gross pathology abnormalities were observed in rats exposed to 50 mg/kg except for a reddened colon in one male and a reddened cervical lymph node in one female. The IV NOAEL for BIA 2-093 in rats is < 50 mg/kg.

Sprague-Dawley Crl:CD(SD) rats in Study 093-843 [redacted] 11/10/2004) were dosed by oral gavage with a single dose of 150, 500, 1000, 2000 mg/kg [redacted] and euthanized 14 days later. Males and females exposed to ≥ 500 mg/kg [redacted] exhibited subdued behavior, staggering and hunched posture. At ≥ 1000 mg/kg animals exhibited labored breathing and piloerection. No animals died during the course of this study. No abnormal clinical signs were observed in animals exposed to 150 mg/kg [redacted]. Decreased BW gain (20-43%) was observed in rats exposed to 1000 or 2000 mg/kg [redacted] when compared to

rats exposed to 150 mg/kg. No gross pathology abnormalities were observed in any rats from any dose group.

Sprague-Dawley Crl:CD (SD) rats in Study 093-844 (b) (4) 11/10/2004) were given a single intravenous dose of 25 or 50 mg/kg (b) (4) and euthanized 14 days later. Abnormal clinical signs such as labored breathing, staggering, subdued behavior and prostration occurred at both doses. These signs did not last beyond the first 24 hours of observation. No gross pathology abnormalities were observed. The NOAEL for an IV bolus of (b) (4) in rats is < 25 mg/kg.

6.2 Repeat-dose toxicity

6.2.1 Mouse

6.2.1. A. Study title: 4 Week Oral (Gavage) Dose Range Finding Study in the Mouse

Key study findings:

- **Liver weight was increased in both male and female mice exposed to BIA 2-093.**
- **Relative kidney weight was increased in all female mice but not in males.**
- **A NOEL was not determined for either effect (<300 mg/kg).**

Study no.: 093-805
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 9/18/2001
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093; Lot 0000012976; Purity= 99.6%
Methods -

Doses: 0, 300 (LD), 400 (MD), 500 (HD) mg/kg
Species/strain: CD-1 mice (b) (4)
Number/sex/group or time point: 5/sex/dose
Route: Once daily by oral gavage
Formulation/vehicle: 0.5% hydroxypropylmethylcellulose (HPMC)
Dose volume/infusion rate: 10 ml/kg
Age/ Weight: 5 weeks old/ 24.8-28.7 g M; 22.1-26.1 g F

Observations times and results:

Mortality & Clinical signs: One HDM was found dead on Day 16 and 2 HDFs were euthanized *in extremis* on Day 1. Piloerection and unsteady gait were observed in mice in all groups on the first day of dosing. These clinical symptoms were observed to occur sporadically through the 1st two dosing days in both males and females. In addition, HDFs exhibited tremors (2/5), hunched posture (1/5), irregular breathing (3/5) and subdued demeanor (3/5).

Body weights: No statistically significant alteration in BW or BW gain was observed in mice exposed to BIA 2-093 when compared to vehicle controls.

Organ weights: Absolute liver weight was increased in all male (LD= 10%, MD= 21%, HD= 26%) and female (LD= 26%, MD= 24%, HD= 42%) dose groups when compared

to controls. In addition, a treatment-related increase in kidney weight, when expressed as a percentage of total body weight, was observed in all female dose group (Relative Kidney Weight: Control= 1.37%, LD= 1.56%, MD= 1.51%, HD=1.59%). Liver weight when expressed as a percentage of body weight was increased in all dose groups in both sexes.

Gross pathology: No treatment related abnormalities were observed in male and female mice exposed to BIA 2-093.

6.2.1. B. Study title: Thirteen Week Oral (Gavage) Dose Range Finding Study in the Mouse

Key study findings:

- **Mortality was observed in both males and females exposed to 650 mg/kg.**
- **Clinical signs were not observed in mice exposed to 150 mg/kg.**
- **Spleen weights were increased in all male dose groups and in females exposed to ≥ 500 mg/kg/day BIA 2-093. Extramedullary hematopoiesis severity in the spleen was increased in a dose dependent manner in males and females exposed to ≥ 350 mg/kg/day BIA 2-093.**
- **All male and female dose groups exposed to BIA 2-093 exhibited increased absolute and relative liver weight. Centrilobular hypertrophy severity was increased in a dose dependent manner in all animals exposed to BIA 2-093.**

Study no.: 093-806
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 12/4/2001
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, Lot 0000012976, Purity= 99.6%
Methods -

Doses: 0, 150 (LD), 350 (MD1), 500 (MD2), 650 (HD) mg/kg/day
Species/strain: CD-1 mice (b) (4)
Number/sex/group or time point: 10/sex/ group; 24 additional /sex/group for TK
Route: Oral gavage
Formulation/vehicle: 0.5% hydroxypropylmethylcellulose (HPMC)
Dosing solution analyses/drug stability and homogeneity: Not performed
Dose volume/infusion rate: 10 ml/kg (0-500 mg/kg); 13 ml/kg (650 mg/kg)
Age: 5 weeks old
Weight: 19.8-24.7 g M; 19.2-24.5 g F

Observations times and results:

Mortality: One male (1/10) and one female (1/10) from the 650 mg/kg dose group were euthanized *in extremis* on Day 6 and Day 5, respectively. The clinical signs in these animals were subdued, hunched posture, piloerection, red discharge from eye. One HDM

(1/24) and one HDF (1/24) from the TK group were also euthanized *in extremis* on Day 6 with symptoms similar to those in the main study group.

Clinical signs: Clinical signs were observed in mice exposed to daily doses of ≥ 350 mg/kg. Most male and female mice exhibited clinical signs on Days 1 and 2 of the study. However, a few mice exhibited symptoms up to day 11 of dosing, after which clinical signs were rarely observed (Reviewer's Table 6.1).

A) Male Mice

Group	Symptom	1	2	3	4	5	6	7	8	9	10	11
350	Subdued	2	-	-	-	-	-	-	-	-	-	-
	Unsteady gait	6	-	-	-	-	-	-	-	-	-	-
500	Subdued	23	-	-	-	-	-	-	-	-	-	-
	Unsteady gait	8	-	-	-	-	-	-	-	-	-	-
	Prostrate	6	-	-	-	-	-	-	-	-	-	-
	Unsteady gait	12	2	2	-	-	-	-	-	-	-	-
650	Subdued	23	12	2	-	2	1	-	1	-	-	-
	Unsteady gait	22	9	9	-	2	2	-	-	2	-	-
	Prostrate	10	1	-	-	-	-	-	-	-	-	-
	Piloerection	-	-	1	1	2	2	1	-	1	-	3
	Tremors	-	-	-	-	1	-	-	-	-	-	-
	Uncoordination	3	2	-	-	-	-	-	-	-	-	-
	Cold body surface	-	-	-	-	1	-	-	-	-	-	-

B) Female Mice

Group	Symptom	1	2	3	4	5	6	7	8	9	10	11
350	Subdued	6	-	-	-	-	-	-	-	-	-	-
	Unsteady gait	8	-	-	-	-	-	-	-	-	-	-
500	Subdued	14	2	1	-	-	-	-	-	-	-	-
	Unsteady gait	17	1	7	-	1	-	-	-	-	-	-
	Prostrate	3	1	2	-	-	-	-	-	-	-	-
	Incoordination	1	-	-	-	-	-	-	-	-	-	-
650	Subdued	26	14	3	-	1	1	-	-	-	1	-
	Unsteady gait	15	8	8	-	2	2	-	-	3	-	-
	Prostrate	14	6	-	-	-	-	-	-	-	-	-
	Piloerection	-	-	1	1	1	-	-	-	-	-	2
	Tremors	-	-	-	-	-	-	-	-	-	1	-
	Incoordination	2	1	-	-	-	-	-	-	-	-	-
	Cold body surface	-	-	-	-	-	-	-	-	-	1	-

Reviewer's Table 6.1: Daily Clinical Signs Observed in Mice Dosed With BIA 2-093. The data presented in the tables are a combination of the animals in the main study and the TK study. Therefore, the total number of animals per group was 34/sex/dose on Day 1; 24/sex/dose on Day 2 and 22/ sex/dose on Day 3. Due to the animals euthanized in extremis, the total number of HDF was 21 on day 5 and 20/sex/group on day 6. A) Male mice B) Female mice.

Body weights: Female mice exposed to BIA 2-093 exhibited increased BW gain compared to the vehicle control group during the course of the study. A similar effect of BIA 2-093 was not observed in males (Reviewer's Table 6.2).

Weight Gain Week 1-13 (g)	0 mg/kg	150 mg/kg	350 mg/kg	500 mg/kg	650 mg/kg
Male	9.7 ± 1.9	9.2 ± 2.7	9.4 ± 2.1	10.7 ± 2.2	10.8 ± 2.5
Female	6.5 ± 2.7	8.5 ± 1.5*	9.0 ± 2.4*	8.6 ± 3.0*	10.1 ± 3.3*

*Reviewer's Table 6.2: Body Weight Gain from Week 1 to Week 13 Was Increased in Female but not Male Mice. *= statistically significantly different from vehicle control.*

Food consumption: No treatment effect was observed on food consumption.

Gross pathology: No treatment-related gross abnormalities were observed during necropsy except for 2/10 HDF that exhibited prominent reticulation of the liver.

Organ weights: Absolute and relative weights of liver were increased in a dose dependent fashion in all males and females exposed to BIA 2-093. The absolute (13-34%) and relative weights of the spleen in all males exposed to BIA 2-093 were also increased in a dose dependent manner. The absolute (~25%) and relative weight of the thymus was decreased in MD2M and HDM. Absolute kidney weight was increased by 10% in HDFs.

Histopathology: Peer review: No; Adequate Battery: No. The following tissues were not collected or examined: mammary gland, Harderian gland, lachrymal gland, nasal cavity/turbinates, tongue, Zymbal gland.

The severity of centrilobular hypertrophy was increased in the liver of both sexes of all treated groups. An increased severity of extramedullary hematopoiesis was observed in both male and female mice exposed to daily doses of ≥ 350 mg/kg. This finding is consistent with the increased weight of spleens observed in males.

	Males					Females				
Dose in mg/kg/day	0	150	350	500	650	0	150	350	500	650
No Examined	10	10	10	10	10	10	10	10	10	10
Liver										
Centrilobular Hypertrophy										
Slight	0	4	1	1	1	0	8	9	8	4
Moderate	0	5	5	4	4	0	0	0	1	5
Marked	0	0	4	5	5	0	0	0	0	0
Total	0	9	10	10	10	0	8	9	9	9
Spleen										
Extramedullary Haemopoiesis										
Slight	10	8	6	6	4	8	6	4	3	5
Moderate	0	0	4	3	6	2	3	5	5	5
Marked	0	0	0	0	0	0	1	1	2	0
Total	10	8	10	9	10	10	10	10	10	10

Sponsor's Table: Histopathology Summary

Toxicokinetics: Sponsor's Tables 25-30 (provided below) present the TK analysis of the parent compound BIA 2-093, S- and R-licarbazepine (BIA 2-005) and OXC. TK parameters besides C_{max} could only be calculated for BIA 2-093 on the first day of the study. This is mainly due to the robust metabolism of BIA 2-093 to BIA 2-194, BIA 2-195 and OXC. Exposure to BIA 2-005 increased in a dose-proportional manner, whereas OXC exposure increased in a greater than dose proportional manner.

Table 25

Toxicokinetic parameters of BIA 2-093 for male and female mice following a single oral administration of BIA 2-093 at 150 to 650 mg/kg/day: Day 1

Males

Dose (mg/kg/day)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	λ_z (/h)	$t_{1/2}$ (h)	R_T
150	33.9	0.500	NC*	NC*	NC*	NC*
350	219	0.500	511	0.411*	1.69*	1.00
500	372	0.500	1641	NC	NC	NC
650	519	0.500	1170	NC	NC	NC

Females

Dose (mg/kg/day)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	λ_z (/h)	$t_{1/2}$ (h)	R_T
150	349	0.500	402	NC	NC	NC
350	273	0.500	1134	0.233	2.97	1.00
500	910	0.500	3934	NC	NC	NC
650	931	0.500	2851	NC	NC	NC

* = Unreliable estimate; only 3 data points used in regression analysis
NC = Not calculated; terminal monoexponential phase could not be unambiguously identified
NC* = Not calculated; only one measurable concentration

Table 26

Toxicokinetic parameters of BIA 2-093 for male and female mice following repeated oral administration of BIA 2-093 at 150 to 650 mg/kg/day: Week 13

Males

Dose (mg/kg/day)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	λ_z (/h)	$t_{1/2}$ (h)	R_O
150	60.3	0.500	NC*	NC*	NC*	NC*
350	25.4	8.00	NC*	NC*	NC*	NC*
500	196	0.500	NC*	NC*	NC*	NC*
650	56.5	0.500	NC*	NC*	NC*	NC*

Females

Dose (mg/kg/day)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	λ_z (/h)	$t_{1/2}$ (h)	R_O
150	30.1	0.500	NC*	NC*	NC*	NC*
350	55.0	0.500	NC*	NC*	NC*	NC*
500	NC#	NC#	NC#	NC#	NC#	NC#
650	29.4	0.500	NC*	NC*	NC*	NC*

NC* = Not calculated; only one measurable concentration
NC# = Not calculated; no measurable concentrations

Table 27

Toxicokinetic parameters of BIA 2-005 for male and female mice following a single oral administration of BIA 2-093 at 150 to 650 mg/kg/day: Day 1

Males

Dose (mg/kg/day)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	λ_z (/h)	$t_{1/2}$ (h)	R_T	Metabolite Ratio
150	40509	0.500	203782	0.363*	1.91*	1.00	NC$
350	38560	0.500	419621	0.253*	2.74*	1.00	821
500	65854	0.500	733517	0.339*	2.05*	1.00	447
650	62095	0.500	783470	0.420*	1.65*	1.00	670

Females

Dose (mg/kg/day)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	λ_z (/h)	$t_{1/2}$ (h)	R_T	Metabolite Ratio
150	37303	0.500	173533	0.392*	1.77*	1.00	432
350	44057	0.500	347879	0.187*	3.70*	1.01	307
500	55791	0.500	605602	0.0385*#	18.0*#	1.66	154
650	70929	0.500	676815	NC	NC	NC	237

* = Unreliable estimate; only 3 data points used in regression analysis
\# = Unreliable estimate; period over which λ_z was determined was less than twice the resultant $t_{1/2}$
NC = Not calculated; terminal monoexponential phase could not be unambiguously identified
NC$ = Not calculated; AUC_{0-24h} was not available for BIA 2-093

Table 28

Toxicokinetic parameters of BIA 2-005 for male and female mice following repeated oral administration of BIA 2-093 at 150 to 650 mg/kg/day: Week 13

Males

Dose (mg/kg/day)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	λ_z (/h)	$t_{1/2}$ (h)	R_O	Metabolite Ratio
150	32991	0.500	136129	0.521*	1.33*	0.668	NC$
350	41071	0.500	244847	0.225	3.08	0.583	NC$
500	81494	0.500	400914	0.140	4.97	0.547	NC$
650	87611	0.500	494377	0.327*	2.12*	0.631	NC$

Females

Dose (mg/kg/day)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	λ_z (/h)	$t_{1/2}$ (h)	R_O	Metabolite Ratio
150	44573	0.500	104138	0.487	1.42	0.600	NC$
350	46218	0.500	147377	0.636*	1.09*	0.424	NC$
500	64099	0.500	296299	NC	NC	0.489	NC$
650	78813	0.500	355324	0.0817*#	8.48*#	0.525	NC$

\# = Unreliable estimate; period over which λ_z was determined was less than twice the resultant $t_{1/2}$
* = Unreliable estimate; only 3 data points used in regression analysis
NC = Not calculated; terminal monoexponential phase could not be unambiguously identified
NC$ = Not calculated; AUC_{0-24h} was not available for BIA 2-093

Table 29

Toxicokinetic parameters of Oxcarbazepine for male and female mice following a single oral administration of BIA 2-093 at 150 to 650 mg/kg/day: Day 1

Male

Dose (mg/kg/day)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	λ_z (/h)	$t_{1/2}$ (h)	R_T	Metabolite Ratio
150	784	0.500	5265	0.203*	3.42*	1.01	NC$
350	1772	8.00	26826	0.239*	2.90*	1.00	52.5
500	3502	4.00	51971	NC	NC	NC	31.7
650	4248	8.00	44568	NC	NC	NC	267

Female

Dose (mg/kg/day)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	λ_z (/h)	$t_{1/2}$ (h)	R_T	Metabolite Ratio
150	2208	2.00	12550	0.346*	2.00*	1.00	31.2
350	4645	2.00	34426	0.190*	3.65*	1.01	30.3
500	5613	4.00	59587	0.194*	3.56*	1.01	15.1
650	6018	12.0	83170	NC	NC	NC	29.2

* = Unreliable estimate; only 3 data points used in regression analysis
NC = Not calculated; terminal monoexponential phase could not be unambiguously identified
NC$ = Not calculated; AUC_{0-24h} was not available for BIA 2-093

Table 30

Toxicokinetic parameters of Oxcarbazepine for male and female mice following repeated oral administration of BIA 2-093 at 150 to 650 mg/kg/day: Week 13

Male

Dose (mg/kg/day)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	λ_z (/h)	$t_{1/2}$ (h)	R_O	Metabolite Ratio
150	556	4.00	3021	0.378*	1.84*	0.574	NC$
350	620	0.500	5441	NC	NC	0.203	NC$
500	1873	0.500	12418	NC	NC	0.239	NC$
650	1656	0.500	20607	NC	NC	0.462	NC$

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Female

Dose (mg/kg/day)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	λ_z (/h)	$t_{1/2}$ (h)	R_O	Metabolite Ratio
150	1845	4.00	9219	0.474*	1.46*	0.735	NC$
350	2710	4.00	12242	NC	NC	0.356	NC$
500	2289	2.00	30391	NC	NC	0.510	NC$
650	3859	0.500	37595	0.150*	4.61*	0.452	NC$

* = Unreliable estimate; only 3 data points used in regression analysis
NC = Not calculated; terminal monoexponential phase could not be unambiguously identified
NC$ = Not calculated; AUC_{0-24h} was not available for BIA 2-093

Sponsor's Table: Toxicokinetics

6.2.2 Rat

6.2.2. A. Study title: 2 Week Oral (Gavage) Dose Finding Study in the Rat

Key study findings:

- **Females exposed to 500 mg/kg were euthanized *in extremis* on Day 2 of the study.**
- **Subdued behavior and unsteady gait were observed at 500 mg/kg.**
- **Distended abdomen was observed in rats exposed to doses greater than 50 mg/kg. Evidence of food in the distended stomachs was observed in the rats euthanized prematurely. This is consistent with the safety pharmacology study which demonstrates that BIA 2-093 decreases GI motility.**
- **Liver weights were increased in all female dose groups and males exposed to doses greater than 50 mg/kg.**
- **Testes weights were increased in all male dose groups. Kidney weights were increased in all female dose groups.**
- **Histopathology was not conducted.**

Study no.: 093-807
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 11/26/1997
GLP compliance: Yes

QA statement: No
Drug, lot #, and % purity: BIA 2-093, Lot PC96.03.22, Purity = 100.7%
Methods

Doses: 0, 50 (LD), 150 (MD1), 300 (MD2), 500(HD) mg/kg, once/day for 14 d
Species/strain: Sprague-Dawley (b) (4)
Number/sex/group or time point: 5/sex/group
Route: Oral gavage
Formulation/vehicle: 0.5% methylcellulose
Dose volume/infusion rate: 5 ml/kg
Age & Weight: 5 weeks old, 127-142 g M; 122-136 g F

Observations times and results:

Mortality & Clinical signs: On day 2, 4/5 HDFs were euthanized *in extremis.* Stomach distension due to solid material resembling food was observed in 3/4 of these animals. In addition, these animals exhibited the following clinical signs before euthanasia: unsteady gait, prostration, partially closed eyes, cold body surface, and piloerection.

No abnormal clinical signs were observed in rats exposed to 50 mg/kg/day. Distended abdomen was observed in 1/5 MD2M, 1/5 HDM ,1/5 MD1F, 1/5 MD2F, and 3/5 HDF within 1-3 hours after dosing. Subdued behavior was observed in 5/5 HDM and 4/5 HDF. Unsteady gait was observed in 5/5 HDM and 5/5 HDF.

Body weights & Food consumption: No statistically significant alteration in body weight gain or food consumption was observed in either males or females exposed to BIA 2-093.

Gross pathology & Organ weights: Besides the distended stomachs observed in the 4/5 HDFs euthanized *in extremis*, there were no abnormal observations in the gross pathology examination.

Absolute and relative liver weights were increased in MD1, MD2, HD males and all female dose groups in a dose dependent manner. Absolute, but not relative, testes weight was increased in all male dose groups. Absolute and relative kidney weights were increased in all female dose groups.

Group sex		Liver# g	Liver# %	Group sex		Liver# g	Liver# %	Testes# g
1M	Mean	12.23	4.839	1F	Mean	6.94	3.810	3.81
	S.D.	2.38	0.682		S.D.	0.52	0.212	0.30
	N	5	5		N	5	5	5
2M	Mean	13.53	5.223	2F	Mean	8.37**	4.651***	4.05*
	S.D.	0.87	0.091		S.D.	0.65	0.337	0.23
	N	5	5		N	5	5	5
3M	Mean	14.93**	5.562**	3F	Mean	9.37***	5.036***	4.11*
	S.D.	2.03	0.487		S.D.	0.72	0.198	0.08
	N	5	5		N	5	5	5
4M	Mean	15.40**	6.030***	4F	Mean	9.76***	5.376***	4.08*
	S.D.	0.62	0.194		S.D.	1.04	0.335	0.17
	N	5	5		N	5	5	5
5M	Mean	16.10**	6.211***	5F	Mean	11.91	6.077	4.16*
	S.D.	1.18	0.322		S.D.	.	.	0.22
	N	5	5		N	1	1	5

Sponsor's Table 3 (excerpted); Study 093-807: Liver and Testes Weights of Male Rats Exposed to BIA 2-093 for 14 days. #= statistically analyzed, *=p<0.05, **=p<0.01, ***=p<0.001.

Group sex		Kidneys# g	Kidneys# %
1F	Mean	1.36	0.750
	S.D.	0.11	0.061
	N	5	5
2F	Mean	1.51*	0.840**
	S.D.	0.06	0.031
	N	5	5
3F	Mean	1.55**	0.836**
	S.D.	0.09	0.027
	N	5	5
4F	Mean	1.52*	0.839**
	S.D.	0.11	0.031
	N	5	5
5F	Mean	1.69	0.862
	S.D.	.	.
	N	1	1

Sponsor's Table 3 (excerpted); Study 093-807: Kidney Weights of Rats Exposed to BIA 2-093 for 14 days. #= statistically analyzed, *=p<0.05, **=p<0.01, ***=p<0.001.

6.2.2. B. Study title: BIA 2-194: 4 Week Preliminary Toxicity Study by Oral (Gavage) Administration to Rats

Key study findings:

- **NOAEL of 75 mg/kg for males and 25 mg/kg for females based on increased liver weights at higher doses.**
- **Increased liver weights associated with centrilobular hypertrophy were observed in rats dosed with ≥ 250 mg/kg.**
- **Females exposed to doses ≥ 250 mg/kg exhibited thyroid epithelial hypertrophy.**
- **Cholesterol was consistently increased in males and females at doses up to 750 mg/kg.**
- **Males dosed with ≥ 250 mg/kg had decreased RBC, Hb and PCV% (hematocrit).**

Study no.: 093-811
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 8/5/2002
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-194, DL020611, purity= 99.6%
Methods -

Doses:

Group number	Colour code	Number of animals		Animal identification numbers		Dose level (mg/kg/day) BIA 2-194				
		Males	Females	Males	Females	Days 1 - 3	Days 4 - 7	Days 8 - 14	Days 15 - 17	Days 18 - 28
1	White	6	6	1 – 6	31 - 36	0	0	0	0	0
2	Green	6	6	7 – 12	37 - 42	25	25	25	25	2000*
3	Yellow	6	6	13 – 18	43 - 48	25	75	75	75	75
4	Blue	6	6	19 – 24	49 - 54	25	75	250	250	250
5	Pink	6	6	25 – 30	55 - 60	25	75	250	750	750

* Males dosed for 2 days, females dosed for 1 day then all euthanased.

Sponsor's Table

Species/strain: HsdBrlHan:Wist strain of rat
Route: Oral gavage
Formulation/vehicle: 0.5% hydroxypropylmethylcellulose
Dosing solution analyses/drug stability and homogeneity: All solutions were within +/- 10% of the nominal concentration except for the bottom of the Group 4 sample and all portions of the Group 5 sample. Reanalysis was not performed due to lack of available samples. Therefore, dosing would be expected to be highly variable in Groups 4 and 5 due to non-homogeneity of the dosing solutions.
Dose volume/infusion rate: 5 ml/kg
Age & Weight: 4-5 weeks; 142-173 g M, 114-135 g F

Observations times and results:

Mortality: All animals exposed to 2000 mg/kg were euthanized *in extremis* on the second day of dosing. Severe symptoms exhibited in these animals before euthanasia were decreased activity, slow breathing, cold body surface, partially closed eyes.

Clinical signs: Clinical signs were not observed at ≤ 250 mg/kg. Rats exposed to 750 mg/kg exhibited extensive salivation.

Body weights & Food consumption: No dose-dependent alteration in BW gain or food consumption was observed in any dose group.

Hematology: Males, but not females, exhibited a decrease in RBC, Hb and PCV%.

Text Table 2 - Group mean haematology parameters expressed as a percentage of Control values

Group/ sex	Dose level (mg/kg/day)	Group mean values for selected haematology parameters and as expressed as a percentage (** %) of Controls		
		RBC (10^{-6}/uL)	Hb (g/dL)	PCV (%)
1M	0	8.59	16.5	48.3
3M	25/75	8.04 (-6 %)	16.1 (-2 %)	46.4 (-4 %)
4M	25/75/250	**8.02 (-7 %)**	**15.2 (-8 %)**	**44.6 (-8 %)**
5M	25/75/250/750	**7.89 (-8 %)**	**15.2 (-8 %)**	**45.1 (-7 %)**
1F	0	7.75	14.9	42.5
3F	25/75	7.79 (+1 %)	15.0 (+1 %)	43.3 (+2 %)
4F	25/75/250	7.55 (-3 %)	14.8 (-1 %)	42.3 (0 %)
5F	25/75/250/750	7.50 (-3 %)	14.3 (-4 %)	41.6 (-2 %)

Values considered to be notable are highlighted in bold.

Sponsor's Table 2: Hematology Analysis

Clinical chemistry: Male rats in group 5 exhibited increased BUN, creatinine, and cholesterol. Group 4 and 5 females also exhibited increased cholesterol. ALT was elevated in these two groups of females as well.

Text Table 3 - Group mean selected blood chemistry parameters expressed as a percentage of Control values

Group/ sex	Dose level (mg/kg/day)	Group mean values for selected blood chemistry parameters and as expressed as a percentage (** %) of Controls					
		Urea (mg/dL)	Creat (mg/dL)	Chol (mg/dL)	ALP (U/L)	ALT (U/L)	AST (U/L)
1M	0	35.7	0.29	85	495	48	72
3M	25/75	35.6 (0 %)	0.28 (-3 %)	85 (0 %)	400 (-19 %)	29 (-34 %)	62 (-14 %)
4M	25/75/250	37.8 (+6 %)	0.30 (+3 %)	98 (+15 %)	422 (-15 %)	35 (-23 %)	83 (+15 %)
5M	25/75/250/750	**42.0 (+18%)**	**0.36 (+24%)**	**124 (+46%)**	**360 (-27 %)**	32 (-33 %)	57 (-21 %)
1F	0	50.9	0.38	75	285	35	65
3F	25/75	44.7 (-12 %)	0.35 (-8 %)	93 (+24 %)	237 (-17 %)	38 (−9)	58 (-11 %)
4F	25/75/250	37.7 (-26 %)	0.31 (-18 %)	**115 (+53 %)**	252 (-12 %)	**61 (+74)**	53 (-18 %)
5F	25/75/250/750	43.2 (-15 %)	0.33 (-13 %)	**122 (+63 %)**	203 (-29 %)	**83 (+137)**	59 (-9 %)

Values considered to be notable are highlighted in bold.

Sponsor's Table 3: Clinical Chemistry Analysis

Gross pathology: Males and females in the 2000 mg/kg/day group had distended stomachs. No other abnormal macroscopic findings were observed.

Organ weights: Increased absolute and mean liver weights were observed in Group 4-5 males (Absolute weight= 14-38%; Relative= 15-39%) and Group 3-5 females (Absolute weight= 21-78%; Relative= 12-67%).

Histopathology: Peer review: Not mentioned

Centrilobular hypertrophy was observed in males (Group 4= 6/6; Group 5= 6/6) and females (Group 4= 5/6; Group 5= 6/6) which correlated with the increase in absolute and relative liver weights in these animals. Centrilobular hypertrophy severity increased in a dose-dependent manner in both genders. Hypertrophy of the thyroid epithelium was observed in females as well (Group 4= 3/6; Group 5= 6/6).

6.2.2. C. Study title: Four Week Oral (Gavage) Repeat Dose Toxicity Study in the Rat

Key study findings:

- **Liver weights were increased in females exposed to ≥ 20 mg/kg and in males exposed to ≥ 75 mg/kg BIA 2-093. Centrilobular hypertrophy was observed in all rats exposed to ≥ 75 mg/kg BIA 2-093.**
- **ALT was elevated slightly in females exposed to ≥ 75 mg/kg BIA 2-093.**
- **Cholesterol was increased in all dose groups of males and females, with the largest increase being in females.**
- **Testes weights were increased in males exposed to ≥ 75 mg/kg BIA 2-093.**
- **Kidney weights were increased in males and females exposed to 250 mg/kg BIA 2-093.**
- **Hematocrit was increased in all male dose groups and MCV was increased in males exposed to ≥ 75 mg/kg BIA 2-093.**
- **However, females exposed to 250 mg/kg/day exhibited a significant decrease in RBC count, increased MCV and a trend for increased reticulocyte number when compared to control. The combination of increased MCV and trend for increased reticulocyte number suggests slight reticulocytosis.**
- **Platelet count was increased in all female dose groups.**

Study no.: 093-808
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 3/9/1998
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, Lot BN980220, Purity = 100.2%
Methods -

Doses: 0, 20 (LD), 75 (MD), 250 (HD) mg/kg
Species/strain: Sprague-Dawley rats (b) (4)
Number/sex/group or time point: 10 per main group and 12 per TK group
Route: Oral gavage
Formulation/vehicle: 0.5% methylcellulose
Dose volume/infusion rate: 5 ml/kg
Age: 5 weeks
Weight: 147-175 g M; 132-161 g F

Observations times and results:

Mortality: None

Clinical signs: No clinical signs were observed at 20 mg/kg. Sporadic salivation and fur staining was observed in the MD and HD rats.

Body weights & Food consumption: No effect of BW gain or food consumption was observed in any of the dose groups.

Ophthalmoscopy: No dose-related abnormalities were observed in the ophthalmology examinations.

Hematology: A dose-dependent increase in hematocrit (all dose groups) and MCV (MD and HD) was observed in males. In HDF, MCV was increased slightly and RBC count was decreased. A dose-dependent increase in platelets was observed in females at all doses. HDFs (3/10) also had elevated reticulocyte counts (5.8, 6.1, 6.6 % RBC) when compared to the highest individual result in controls (4.7% RBC).

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Group sex		HCT# %	MCV# fl
1M	Mean	47.1	58.1
	S.D.	2.3	3.8
	N	10	10
2M	Mean	48.5*	59.4
	S.D.	1.4	1.2
	N	10	10
3M	Mean	48.7**	60.6*
	S.D.	0.9	0.9
	N	10	10
4M	Mean	49.4**	61.2**
	S.D.	0.7	0.7
	N	10	10

- statistica .05 **=p<0.01 ***=p<0.0

Group sex		RBC# 10^6/ul	MCV# fl	Plate# 10^3/ul
1F	Mean	8.02	59.3	654
	S.D.	0.39	0.7	114
	N	10	10	10
2F	Mean	8.07	59.7	740*
	S.D.	0.37	0.9	123
	N	10	10	10
3F	Mean	7.78	59.6	816**
	S.D.	0.27	1.2	117
	N	10	10	10
4F	Mean	7.70*	60.6**	868***
	S.D.	0.25	0.9	85
	N	10	10	10

- statistical .01 ***=p<

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Sponsor's Table 4 (excerpted): Hematology in Rats Exposed to BIA 2-093 for 4 Weeks. Group 1= controls, group 2= LD, group 3= MD, group 4 = HD. M=males, F=females, #= statistically analyzed, *=p<0.05, **=p<0.01, ***=p<0.001.

Clinical chemistry: Although cholesterol was elevated in all dose groups in both sexes when compared to vehicle controls, the increase was larger in females. Total protein was elevated in all male dose groups and in the HDF which again had a greater increase than males. Blood calcium was decreased and sodium was increased in all males, in a dose-dependent manner. MDF and HDF, but not males, exhibited a small increase in plasma ALT.

Group sex		Creat# umol/l	AST# U/l	T. Prot# g/l	Chol# mmol/l	Ca# mmol/l	Na# mmol/l
1M	Mean	44	101	65	2.2	2.90	144
	S.D.	3	11	2	0.6	0.12	2
	N	10	10	10	10	10	10
2M	Mean	47	96	67*	2.9***	2.75***	146*
	S.D.	17	6	3	0.5	0.07	1
	N	10	10	10	10	10	10
3M	Mean	40**	93*	68**	2.9***	2.79***	146*
	S.D.	2	5	2	0.4	0.06	0
	N	10	10	10	10	10	10
4M	Mean	42*	92**	68**	3.5***	2.74***	146*
	S.D.	2	6	1	0.4	0.07	1
	N	10	10	10	10	10	10

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Sponsor's Table 5 (excerpted): Male Rat Clinical Chemistry. Group 1= controls, group 2= LD, group 3= MD, group 4 = HD. M=males, #= statistically analyzed, *=p<0.05, **=p<0.01, ***=p<0.001.

Group sex		ALT# U/l	T. Prot# g/l	Alb# g/l	Glob# g/l	Chol# mmol/l
1F	Mean	76	66	31	35	2.7
	S.D.	12	3	2	2	0.5
	N	10	10	10	10	10
2F	Mean	77	67	32	35	3.0*
	S.D.	11	2	1	1	0.4
	N	10	10	10	10	10
3F	Mean	87*	68	32	36	3.3***
	S.D.	17	3	1	2	0.3
	N	10	10	10	10	10
4F	Mean	96**	72***	33**	39***	4.3***
	S.D.	16	4	1	3	0.2
	N	10	10	10	10	10

Sponsor's Table 5 (excerpted): Female Rat Clinical Chemistry. Group 1= controls, group 2= LD, group 3= MD, group 4 = HD. F=females, #= statistically analyzed, *=p<0.05, **=p<0.01, ***=p<0.001.

Urinalysis: No dose related abnormalities were observed on urinalysis.

Gross pathology: No dose-related abnormalities were observed during the necropsy.

Organ weights: Absolute and relative liver weights were increased in MDM, HDM and all female dose groups. Absolute and relative kidney weights were increased in both HD males and females. MDM and HDM had increased absolute testes weight. Relative and absolute adrenal weights were increased in HDM.

Group sex		Liver# g	Adrenals# g	Kidneys# g	Testes# g	Liver# %	Adrenals# %	Kidneys# %
1M	Mean	13.96	0.045	2.18	4.47	4.299	0.0138	0.674
	S.D.	2.02	0.007	0.20	0.21	0.544	0.0022	0.069
	N	10	10	10	10	10	10	10
2M	Mean	15.02	0.049	2.33	4.62	4.524	0.0146	0.701
	S.D.	1.37	0.006	0.17	0.25	0.365	0.0020	0.034
	N	10	10	10	10	10	10	10
3M	Mean	15.91**	0.047	2.34	4.67*	4.759*	0.0141	0.701
	S.D.	2.01	0.005	0.23	0.21	0.501	0.0020	0.065
	N	10	10	10	10	10	10	10
4M	Mean	17.14***	0.052**	2.46**	4.69*	5.278***	0.0161*	0.758**
	S.D.	1.42	0.006	0.23	0.24	0.191	0.0018	0.035
	N	10	10	10	10	10	10	10

Sponsor's Table 7: Organ weights from Male Rats. Group 1= controls, group 2= LD, group 3= MD, group 4 = HD. M=males, #= statistically analyzed, *=p<0.05, **=p<0.01, ***=p<0.001.

Group sex		Liver# g	Kidneys# g	Liver# %	Kidneys# %
1F	Mean	8.44	1.55	3.835	0.703
	S.D.	0.68	0.11	0.180	0.041
	N	10	10	10	10
2F	Mean	8.93*	1.52	4.062*	0.691
	S.D.	0.59	0.12	0.209	0.044
	N	10	10	10	10
3F	Mean	9.63***	1.61	4.290***	0.716
	S.D.	0.48	0.14	0.208	0.058
	N	10	10	10	10
4F	Mean	11.84***	1.69*	5.141***	0.736
	S.D.	1.36	0.18	0.236	0.030
	N	10	10	10	10

Sponsor's Table 7: Organ weights in Female Rats. Group 1= controls, group 2= LD, group 3= MD, group 4 = HD. F=females, #= statistically analyzed, *=p<0.05, **=p<0.01, ***=p<0.001.

Histopathology: Peer review: Yes; Adequate Battery: No. The following tissues were not analyzed: Harderian gland, lachrymal gland, fallopian tubes, nasal cavity, turbinates, rectum, tongue, Zymbal gland.

A dose-dependent increase in centrilobular hypertrophy was observed in MD (1/10 males; 2/10 females) and HD (7/10 males; 7/10 females) rats.

Toxicokinetics: Consistent with previous descriptions of metabolism of BIA 2-093 in the rat, the major metabolite observed in this study was OXC and not BIA 2-194 (called BIA 2-005 in this study due to the inability of the Sponsor to perform chiral analysis at this point in the drug development process). Plasma levels of OXC increased considerably between the first and last day of the study in females but not in males. Production of both metabolites was relatively dose-proportional in females at the end of the study. In males, production of BIA 2-005 was greater than dose proportional between 20 and 75 mg/kg but was dose-proportional between 75 and 250 mg/kg. Production of OXC was dose-proportional in males.

BIA 2-005 (20 mg/kg)

		AUC_{last} (ng.h/ml)	$AUC_{0-\infty}$ (ng.h/ml)	AUC_{0-24h} (ng.h/ml)	C_{max} (ng/ml)	t_{max} (h)	$t_{1/2}$ (h)	λ_z (/h)	R_o
Female	Day 1	1213	1989@	1869	559	0.500	6.56*#	0.106*#	NC
Female	Day 25	1372	2780@	2204	527	0.500	9.38*#	0.0739*#	1.18
Male	Day 1	521	NC	667	372	0.500	NC	NC	NC
Male	Day 25	407	NC	495	331	0.500	NC	NC	NC†

BIA 2-005 (75 mg/kg)

		AUC_{last} (ng.h/ml)	$AUC_{0-\infty}$ (ng.h/ml)	AUC_{0-24h} (ng.h/ml)	C_{max} (ng/ml)	t_{max} (h)	$t_{1/2}$ (h)	λ_z (/h)	R_o
Female	Day 1	3317	NC	7006	736	0.500	NC	NC	NC
Female	Day 25	3971	NC	7879	998	0.500	NC	NC	1.12
Male	Day 1	3211	NC	5943	896	0.500	NC	NC	NC
Male	Day 25	1892	2657@	2831	734	0.500	4.52*#	0.153*#	0.476

BIA 2-005 (250 mg/kg)

		AUC_{last} (ng.h/ml)	$AUC_{0-\infty}$ (ng.h/ml)	AUC_{0-24h} (ng.h/ml)	C_{max} (ng/ml)	t_{max} (h)	$t_{1/2}$ (h)	λ_z (/h)	R_o
Female	Day 1	18034	NC	18034	2889	0.500	NC	NC	NC
Female	Day 25	18271	28841@	18271	3216	0.500	18.3*#	0.0379*#	1.01
Male	Day 1	12630	NC	12630	969	0.500	NC	NC	NC
Male	Day 25	3300	5953@	5265	1132	0.500	7.49*#	0.0926*#	0.417

OXC (20 mg/kg)

		AUC_{last} (ng.h/ml)	$AUC_{0-\infty}$ (ng.h/ml)	AUC_{0-24h} (ng.h/ml)	C_{max} (ng/ml)	t_{max} (h)	$t_{1/2}$ (h)	λ_z (/h)	R_o
Female	Day 1	32672	35074	32672	4274	0.500	6.25	0.111	NC
Female	Day 25	54526	60015	54526	5608	0.500	7.02*	0.0987*	1.67
Male	Day 1	9200	11468	12949	2687	1.00	3.35*#	0.207*#	NC
Male	Day 25	7592	9149	10236	2773	0.500	3.27*#	0.212*#	0.790

OXC (75 mg/kg)

		AUC_{last} (ng.h/ml)	$AUC_{0-\infty}$ (ng.h/ml)	AUC_{0-24h} (ng.h/ml)	C_{max} (ng/ml)	t_{max} (h)	$t_{1/2}$ (h)	λ_z (/h)	R_o
Female	Day 1	168542	NC	168542	11128	0.500	NC	NC	NC
Female	Day 25	194837	NC	194837	14864	0.500	NC	NC	1.16
Male	Day 1	64276	NC	64276	7523	0.500	NC	NC	NC
Male	Day 25	31836	33340	31836	7368	0.500	5.68*	0.122*	0.495

OXC (250 mg/kg)

		AUC_{last} (ng.h/ml)	$AUC_{0-\infty}$ (ng.h/ml)	AUC_{0-24h} (ng.h/ml)	C_{max} (ng/ml)	t_{max} (h)	$t_{1/2}$ (h)	λ_z (/h)	R_o
Female	Day 1	312207	NC	312207	15424	0.500	NC	NC	NC
Female	Day 25	381306	NC	381306	25795	0.500	NC	NC	1.22
Male	Day 1	158058	NC	158058	10910	8.00	NC	NC	NC
Male	Day 25	69121	72317	69121	12277	0.500	5.57*	0.124*	0.437

* - unreliable estimate; only 3 values used to calculate λ_z

- unreliable estimate; half-life is greater than twice the interval over which it was calculated

@ - extrapolated area > 20%

NC – not calculated

† - R_o was not calculated because AUC_{0-24h} values on Day 1 and Day 25 were considered unreliable as they were calculated from only 3 data points

Sponsor's Table: Toxicokinetics

6.2.2. D. Study title: Three Month Oral (Gavage) Repeat Dose Toxicity Study in the Rat with a Four Week Recovery Period

Key study findings:

- **NOAEL in this study is 20 mg/kg/day.**
- **Reticulocytosis in male and female rats dosed with ≥ 75 mg/kg may be regenerative in nature since it coincides with a decrease in erythrocytes. At the end of the 4 week recovery period, reticulocytes were still elevated in females and slightly elevated in males.**
- **An increase in platelet counts in all female dose groups at weeks 6 and 13, resolves by the end of the 4 week recovery period.**
- **Cholesterol is significantly increased throughout the dosing period in males and females dosed with ≥ 75 mg/kg. The increase resolves to normal in all groups but males exposed to 250 mg/kg.**
- **The kidneys are a major target of BIA 2-093 in rats. Kidney weight was elevated in a dose-dependent manner in all male and female groups. The incidence of nephropathy in females and the severity of nephropathy in males exposed to ≥ 75 mg/kg BIA 2-093 was increased in a dose-dependent manner. A dose-dependent increase in the incidence of leukocyte presence was also observed in the urine of females. Nephropathy was still observed in BIA 2-093 exposed rats at the end of the 4 week recovery period.**
- **Increased liver weights, that did not resolve in the rats exposed to 250 mg/kg, were observed in males exposed to 250 mg/kg and females dosed with ≥ 75 mg/kg. Centrilobular hypertrophy was observed in these dose groups.**
- **Thyroid follicular hypertrophy was observed in all dose groups in both sexes in a dose-dependent manner but was not observed in animals from the recovery group.**
- **As with previous studies involving dosing of rats with BIA 2-093, OXC was observed to be the major metabolite formed *in vivo*.**

Study no.: 093-809
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 10/27/2000
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, Lot 0000012976, Purity = 99.6%
Methods -

Doses: 0, 20 (LD), 75 (MD), 250 (HD) mg/kg/day
Species/strain: Sprague-Dawley rats (b) (4)
Number/sex/group or time point (main study): 15 main study; 12 in TK groups.
Route: Oral gavage
Formulation/vehicle: 0.5% hydroxypropylmethylcellulose
Dosing solution analyses/drug stability and homogeneity: Since the dosing solutions were suspensions, it was important to determine the homogeneity of the solutions. Except for

samples being out of specification on Day 3 and Day 13, samples were determined to be within the acceptable criteria for homogeneity.
Dose volume/infusion rate: 5 ml/kg
Age: 5 weeks
Weight: 108-128 g M; 101-133 g F

Observations times and results:

Mortality: One HDM was euthanized *in extremis* on Day 53 due to severe clinical signs such as cold body surface, subdued behavior and rapid breathing.

Clinical signs: Fur staining, hair loss on the neck and scabbing on the neck were the most common clinical signs observed during the study. These signs were not observed in a dose-dependent manner. Besides the HDM euthanized on Day 53, only 2 other HDMs on Day 1 exhibited typical clinical signs associated with exposure to BIA 2-093, such as unsteady gait and subdued behavior.

Body weights: BW gain was not altered by BIA 2-093 exposure in males and females during the three month dosing period. In the recovery period, male BW gain was normal; BW gain was decreased in HDFs only (9.6 g in controls vs. 1.0 g in HDFs).

Food consumption: Food consumption was slightly increased in treated males and females during the dosing period, but not during the recovery portion of the study. This increase was on the average about 1-3 g more than the control animals.

Ophthalmoscopy: No line listings of the opthalmoscopic analysis were provided in this study report. The analysis of the consulting veterinarian states that no test article-related abnormalities were observed.

Hematology: As was observed in Study 093-808, platelet count was increased in all female, but not male, dose groups at week 6 (15-24%) and week 13 (8-14%), but not in a dose-proportional manner. Platelet count was normal in all dose groups during the recovery period.

At week 6, HD males exhibited an increase in MCV and MD and HD males exhibited an increase in MCH. This dose-dependent increase persisted into week 13 and was observed in all male dose groups at this time point. The increase in MCV and MCH at week 13 was accompanied by a slight but significant decrease in RBC count and a corresponding increase in the number reticulocytes in the MD and HD group. Similar changes were observed in MDF and HDF at weeks 13 and 17. The increase in reticulocyte number did not resolve by the end of the 4 week recovery period in females and was slightly, but not statistically significantly, increased in males.

Group sex		Week 6 RBC# 10^6/ul	Week 6 MCV# fl	Week 6 MCH# pg	Week 13 RBC# 10^6/ul	Week 13 MCV# fl	Week 13 MCH# pg	Week 13 Retics# %RBC
1M	Mean	8.22	60.3	19.4	8.60	54.3	17.7	1.0
	S.D.	0.21	1.6	0.5	0.25	1.2	0.5	0.4
	N	10	10	10	9	9	9	9
2M	Mean	8.22	61.1	19.6	8.63	56.6***	18.4***	1.4
	S.D.	0.23	2.0	0.5	0.28	0.9	0.4	0.5
	N	10	10	10	10	10	10	10
3M	Mean	8.09	61.5	19.9*	8.23**	57.6***	18.8***	1.9**
	S.D.	0.29	1.3	0.5	0.28	1.7	0.5	0.8
	N	10	10	10	9	9	9	9
4M	Mean	8.04	61.9*	19.9*	8.25**	57.2***	18.6***	2.0**
	S.D.	0.25	1.5	0.4	0.21	1.3	0.3	0.8
	N	10	10	10	10	10	10	10

Sponsor's Table 3 (excerpted): Hematology in Male Rats. Group 1= controls, group 2= LD, group 3= MD, group 4 = HD. M=males, F=females. #= statistically analyzed; *=p<0.05, **=p<0.01, ***=p<0.001.

Group sex		Week 13 RBC# 10^6/ul	Week 13 Hb# g/dl	Week 13 HCT# %	Week 13 Retics# %RBC	Week 17 MCHC# g/dl	Week 17 Retics# %RBC
1F	Mean	8.15	15.4	46.4	1.2	32.2	1.0
	S.D.	0.30	0.6	1.8	0.4	0.5	0.4
	N	10	10	10	10	5	5
2F	Mean	8.18	15.2	45.7	2.0*	31.8	1.1
	S.D.	0.39	0.5	1.8	0.9	0.3	0.4
	N	10	10	10	10	5	5
3F	Mean	7.81**	14.9*	44.8*	2.6***	31.3**	2.0**
	S.D.	0.23	0.5	1.6	0.7	0.3	0.4
	N	10	10	10	10	5	5
4F	Mean	7.60***	14.4***	43.5***	2.5***	31.4**	1.9**
	S.D.	0.26	0.3	1.4	0.8	0.4	0.3
	N	10	10	10	10	5	5

Sponsor's Table 3 (excerpted): Hematology in Female Rats. Group 1= controls, group 2= LD, group 3= MD, group 4 = HD. M=males, F=females. #= statistically analyzed; *=p<0.05, **=p<0.01, ***=p<0.001.

Clinical chemistry: The most consistent BIA 2-093-related change in blood chemistry parameters was a dose-dependent increase in cholesterol in MDM and HDM and females at week 6 and 13. LDM also exhibited an increase in cholesterol at week 13. This increase in cholesterol was still present in HDM at the end of the recovery period.

Male		Week 6	Week 13	Week 17	Female		Week 6	Week 13
Group sex		Chol# mmol/l	Chol# mmol/l	Chol# mmol/l	Group sex		Chol# mmol/l	Chol# mmol/l
1M	Mean	2.4	2.5	2.5	1F	Mean	3.1	3.2
	S.D.	0.3	0.3	0.4		S.D.	0.5	0.4
	N	10	9	5		N	10	10
2M	Mean	2.5	2.7*	2.6	2F	Mean	3.4	3.3
	S.D.	0.3	0.2	0.1		S.D.	0.3	0.4
	N	10	11	5		N	10	10
3M	Mean	2.9***	2.8*	2.8	3F	Mean	3.6*	3.7*
	S.D.	0.2	0.3	0.4		S.D.	0.5	0.6
	N	10	10	5		N	10	10
4M	Mean	3.2***	3.2***	3.3***	4F	Mean	4.5***	4.5***
	S.D.	0.4	0.3	0.3		S.D.	0.6	0.5
	N	10	10	5		N	10	10

Sponsor's Table 4: Cholesterol Levels in Male and Female Rats. Group 1= controls, group 2= LD, group 3= MD, group 4 = HD. M=males, F=females. #= statistically analyzed; *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$.

Urinalysis: At all time points there was a dose-dependent, qualitative increase in leukocytes observed in the urine of females when compared to controls. Although leukocytes were observed in the urine of all male dose groups, including controls, there was no clear dose-dependent trend in the incidence or severity. The increase in urinary leukocytes persisted in the HDF at the end of the recovery period. Electrolyte concentrations were altered in dose groups when compared to controls but the large variability in values prevents any meaningful analysis of the data.

Gross pathology: MDM (1/10) and HDM (1/10) exhibited mottled kidneys and abnormalities in the liver such as prominent reticulation (1/10 HD males).

Organ weights: HDM exhibited an increase in relative (27%) and absolute (30%) liver weights. Absolute kidney weight was increased in MDM (5%) and HDM (16%) while the relative weight was increased in all male dose groups compared to control (4%, 5%, 14%, LD, MD, HD respectively). Absolute thyroid weight was slightly decreased (4-12%) in a dose dependent manner and heart (5-11%) and kidney (6-13%) absolute weights were both increased in a dose dependent manner in all female dose groups. Absolute liver weights were increased in MDF (26%) and HDF (63%). HDF had decreased thymus weights (32%).

At the end of the recovery period, HDM (4.0%) and HDF (4.2%) exhibited an increase in relative liver weight. MDF (0.7%) and HDF (0.7%) also exhibited a persistent increase in relative kidney weight.

Histopathology: Peer review: Yes; Adequate Battery: No. The following tissues were not examined: Harderian gland, lachrymal gland, nasal cavity/ turbinates, Zymbal gland.

Centrilobular hypertrophy was observed in MDM (2/10), HDM (7/10), MDF (6/10) and HDF (10/10). This observation is consistent with the increased liver weights observed in the HDM, MDF and HDF. The incidence of nephropathy was increased in

MDF (7/10) and HDF (7/10) and the severity was increased in HDM when compared to controls. MDM (5/10) and HDM (5/10) also exhibited hyaline droplet deposition in the kidneys. Thyroid follicular hypertrophy was increased in a dose-dependent manner in males (0/10, 3/10, 8/10, 7/10, in C, LD, MD, HD, respectively) and females (1/10, 1/10, 5/10, 6/10, in C, LD, MD, HD, respectively). Two HDF exhibited focal epithelial hyperplasia of the stomach. All of these findings, except for the kidney nephropathy, were reversible.

Toxicokinetics: As in other studies that examined the TK of BIA 2-093 in rats, this study demonstrates that the major metabolite of BIA 2-093 produced in this species is OXC, not BIA 2-005.

Toxicokinetic parameters of BIA 2-005 for male and female rats following a single oral administration of BIA 2-093 at 20 to 250 mg/kg/day: Day 1

Males

Dose	C_{max} (ng/ml)	t_{max} (h)	$AUC_{0\text{-}24h}$ (ng.h/ml)	$t_{1/2}$ (h)	λ_z (/h)
20 mg/kg	725	0.500	1664	NC	NC
75 mg/kg	1779	0.500	9719	6.20#	0.112#
250 mg/kg	4319	0.500	27910	5.98*	0.116*

Females

Dose	C_{max} (ng/ml)	t_{max} (h)	$AUC_{0\text{-}24h}$ (ng.h/ml)	$t_{1/2}$ (h)	λ_z (/h)
20 mg/kg	518	0.500	1272	NC	NC
75 mg/kg	1028	0.500	12573	NC	NC
250 mg/kg	4928	0.500	20949	NC	NC

\# = Unreliable estimate; period over which λ_z was determined was less than twice the resultant $t_{½}$
* = Unreliable estimate; only 3 data points used in regression analysis
NC = Not calculated; terminal monoexponential phase could not be unambiguously identified

Sponsor's Table 19: BIA 2-005 at Day 1

Toxicokinetic parameters of BIA 2-005 for male and female rats following repeated oral administration of BIA 2-093 at 20 to 250 mg/kg/day: Week 13

Males

Dose	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	$t_{1/2}$ (h)	λ_z (/h)	R_O
20 mg/kg	462	0.500	837	NC	NC	0.503
75 mg/kg	1326	0.500	6723	NC	NC	0.692
250 mg/kg	1729	0.500	19829	NC	NC	0.710

Females

Dose	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	$t_{1/2}$ (h)	λ_z (/h)	R_O
20 mg/kg	618	0.500	3558	NC	NC	2.80
75 mg/kg	2195	0.500	12462	NC	NC	0.991
250 mg/kg	2367	0.500	22365	NC	NC	1.07

NC = Not calculated; terminal monoexponential phase could not be unambiguously identified

Sponsor's Table 20: BIA 2-005 at Week 13

Toxicokinetic parameters of Oxcarbazepine for male and female rats following a single oral administration of BIA 2-093 at 20 to 250 mg/kg/day: Day 1

Males

Dose	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	$t_{1/2}$ (h)	λ_z (/h)
20 mg/kg	2453	0.500	5273	2.54	0.273
75 mg/kg	4169	2.00	34394	NC	NC
250 mg/kg	12618	0.500	82741	4.14*	0.168*

Females

Dose	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	$t_{1/2}$ (h)	λ_z (/h)
20 mg/kg	3664	0.500	13781	NC	NC
75 mg/kg	4933	0.500	53476	NC	NC
250 mg/kg	12810	0.500	88543	NC	NC

* = Unreliable estimate; only 3 data points used in regression analysis
NC = Not calculated; terminal monoexponential phase could not be unambiguously identified

Sponsor's Table 21: OXC at Day 1

Toxicokinetic parameters of Oxcarbazepine for male and female rats following repeated oral administration of BIA 2-093 at 20 to 250 mg/kg/day: Week 13

Males

Dose	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	$t_{1/2}$ (h)	λ_z (/h)	R_O
20 mg/kg	2992	0.500	10836	4.05	0.171	2.06
75 mg/kg	4819	0.500	41204	NC	NC	1.20
250 mg/kg	6524	0.500	80996	NC	NC	0.979

Females

Dose	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	$t_{1/2}$ (h)	λ_z (/h)	R_O
20 mg/kg	3658	0.500	55934	7.45*	0.0930*	4.06
75 mg/kg	9781	0.500	108263	11.6#	0.0599#	2.02
250 mg/kg	9753	4.00	155841	NC	NC	1.76

\# = Unreliable estimate; period over which λ_z was determined was less than twice the resultant $t_{½}$
* = Unreliable estimate; only 3 data points used in regression analysis
NC = Not calculated; terminal monoexponential phase could not be unambiguously identified

Sponsor's Table 22: OXC at Week 13

6.2.2. E. Study title: Three Month Oral (Gavage) Repeat Dose Comparative Toxicity Study in the Han Wistar Rat

Key study findings:

- **Due to the study design, rats from the 300 mg/kg BIA 2-194 and BIA 2-195 dose group were always sampled for non-terminal analyses two weeks after the control animals. Therefore, it is difficult to determine conclusively in these groups if the observations made in these animals are different from controls.**
- **NOAELs for BIA 2-093 and BIA 2-194 were not established in this study. The NOAEL for BIA 2-195 in this study was 75 mg/kg.**
- **Clinical signs in rats exposed to > 300 mg/kg/day BIA 2-093 were consistent with those observed in previous studies. Clinical signs were of lesser severity and incidence in rats exposed to > 300 mg/kg/day BIA 2-194. On the whole, there were few incidents of clinical signs in rats exposed to > 300 mg/kg BIA 2-195.**
- **Slight reticulocytosis and a decrease in RBC count was observed at week 6 in females exposed to 300 mg/kg BIA 2-093, BIA 2-194 (week 8), and 250 and 300 (week 8) mg/kg BIA 2-195. The reticulocytosis in this study was not as robust as was previously observed in the three month study using Sprague-Dawley rats.**
- **APTT was increased throughout the study in females exposed to 300 mg/kg BIA 2-093.**
- **Consistent with previous studies, cholesterol levels were elevated throughout the study in rats exposed to 300 mg/kg BIA 2-093 and BIA 2-194.**
- **Nephropathy, consisting of hyaline droplet accumulation, elevated urinary protein, and basophilic tubules, was evident in male rats dosed with 300 mg/kg BIA 2-093, BIA 2-194 or BIA 2-195. The potency of the compounds for causing**

nephropathy was BIA 2-093> BIA 2-194> BIA 2-195. Females exhibited rare incidences of basophilic tubules and decreased urine volume in BIA 2-093 and BIA 2-194 dose groups.

- **Increased liver weight and hepatic centrilobular hypertrophy were observed in all dose groups of both sexes except for rats exposed to 75 mg/kg/day BIA 2-195. Severity and incidence of the centrilobular hypertrophy was the least in animals exposed to BIA 2-195.**
- **Hepatocyte vacuolization with lipid inclusions was observed in all groups exposed to 300 mg/kg/day of their respective test article.**
- **Thyroid follicular hypertrophy was observed in dose groups exhibiting hepatic centrilobular hypertrophy.**
- **An increase in gastric fundic dilated glands was observed in all female dose groups except for 75 mg/kg BIA 2-195.**
- **Interconversion of BIA 2-194 to BIA 2-195 is minimal in rats. Conversion of BIA 2-093 and BIA 2-194, but not BIA 2-195, to OXC is robust in rats.**

Study no.: 093-813
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 2/10/2005
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-194 (lot 2070-2-1, purity=100.3 %), BIA 2-195 (lot PC041110, purity=100 %), BIA 2-093 (lot 4674-2-4, purity=99%).
Methods –

Doses:

Group	Test / comparator substance	Days / dose level (mg/kg/day)							
		1-3	4-7	8-14	15	16-20	21-25	26/27	28-108 /120†
1	Vehicle	0	0	0	0	0	0	0	0
2	BIA 2-093	25	75	250	750	300	300	300	300
3	BIA 2-194	25	75	250	750	500	500	**	300
4	BIA 2-195	25	75	75	75	75	75	75	75
5	BIA 2-195	25	75	250	250	250	250	250	250
6	BIA 2-195	25	75	250	750	500	500	**	300
7	Vehicle	0	0	0	0	0	0	0	0
8	BIA 2-093	25	75	250	750	300	300/400*	300/400*	300
9	BIA 2-194	25	75	250	750	500	500	**	300
10	BIA 2-195	25	75	75	75	75	75	75	75
11	BIA 2-195	25	75	250	250	250	250	250	250
12	BIA 2-195	25	75	250	750	500	500	**	300

*Four males and 4 females (Animals 808-815) received 400 mg/kg/day from Days 21 to 27.
**Not dosed.
†The study continued up to Day 120 for Groups 3/9 and 6/12 only, to ensure that these groups achieved 13 weeks at the target dose.

Sponsor's Summary Table

Species/strain: HsdBrlHan:Wis, Han Wistar Rats (b) (4)
Number/sex/group or time point: 6/dose/sex main study; 8/dose/sex TK study
Route: Oral gavage
Formulation/vehicle: 0.5% hydroxypropylmethylcellulose

Dosing solution analyses/drug stability and homogeneity: All dosing formulations were within +/- 15% of the nominal concentration.
Age: 8 weeks
Weight: 205-245 g M; 189-233 g F

Observations times and results:

Mortality: In total, 2/14 females exposed to 500 mg/kg/day BIA 2-195 were euthanized on Day 24. One female (1/14) exposed to 750 mg/kg/day BIA 2-194 on Day 15 and one female (1/14) exposed to 750 mg/kg/day BIA 2-195 on Day 15 were euthanized *in extremis* due to severe clinical symptoms.

Clinical signs: Clinical signs in animals that were euthanized *in extremis* were consistent with previously observed signs and consisted of subdued behavior, hunched posture, piloerection, BW loss, partially closed eyes, unsteady gait and hair loss.

There were no clinical signs in animals exposed to doses of the three compounds ≤ 250 mg/kg in the time period between day one and day 15 of the study. On day 15 when the daily dose of BIA 2-093, BIA2-194, or BIA 2-195 was increased to 750 mg/kg/day, clinical signs were observed. The most severe clinical signs were observed in animals exposed to 750 mg/kg BIA 2-093. These signs consisted of hunched posture, subdued behavior, pale body color, cold body surface, piloerection, distended and firm abdomen, irregular breathing, dilated pupils, abnormal gait and uncoordination. A change in demeanor, defined as head rising and continuous air sniffing were also observed in 4 of the females in this group. Similar clinical signs, occurring at a lesser incidence, were noted in females exposed to 750 mg/kg/day BIA 2-194 but not in males. Little to no clinical signs were observed in males and females exposed to 750 mg/kg BIA 2-195. Due to the clinical signs observed in some dose groups exposed to 750 mg/kg, the dose was eventually reduced to 300 mg/kg BIA 2-093, BIA 2-194 and BIA 2-195 for the three month dosing portion of the study. No marked clinical signs were observed in any of the dose groups once the final dose was set at 300 mg/kg for each test article.

Body weights: While there were periods of slight BW loss during the dose adjustment period of the study (Day 1-27), no overall difference in BW gain between week 1 and week 16 was observed in animals dosed with BIA 2-093, BIA 2-194 or BIA 2-195 when compared to controls.

Food consumption: Lower food intake was observed during the dose adjustment period (day 1-28) in females. However, after Day 28 group food intake was similar to controls.

Hematology: At week 8, the group mean for reticulocytes was increased (~25%) in female rat dose groups receiving either 300 mg/kg/day BIA 2-194 or BIA 2-195. A slight increase (~21%) that was not statistically significant was observed in rats exposed to 300 mg/kg BIA 2-093. Coincident with the increase in reticulocytes in these groups was a slight increase in MCH (~4-9%). RBC count was also slightly decreased in females exposed to 250 or 300 mg/kg/day BIA 2-195 and 300 mg/kg/day BIA 2-194 (7%, 7%, and 6%, respectively). A similar overall trend and magnitude of change in reticulocytes, MCH, MCV and RBC was observed at week 15/ week 17 but did not reach statistical

significance. An elevation of APTT (Week 6= 45%; Week 15= 99%) was detected in female rats exposed to 300 mg/kg BIA 2-093, but not in any other dose group.

Clinical chemistry: ALT levels were increased in females exposed to 250 mg/kg/day BIA 2-195 and 300 mg/kg/day BIA 2-093 at week 6 (51 and 57 U/l, respectively; compared to 36 U/l in controls). An increase was also observed in females exposed to 300 mg/kg/day at week 8 (58 U/l). Increased ALT was not observed at any other time point in the study in these dose groups.

Cholesterol levels, as demonstrated in other studies conducted by the Sponsor, were increased in males exposed to 300 mg/kg BIA 2-093 at week 6 (2.0 ± 0.2 mmol/l vs. 1.3 ± 0.3 mmol/l in controls) and in males (1.9 ± 0.4 mmol/l vs. 1.3± 0.2 mmol/l in controls) and females (2.8 ± 0.5 mmol/l vs. 1.9 ± 0.1 mmol/l in controls) exposed at week 15. Cholesterol may have been elevated in females and males exposed to 300 mg/kg/day BIA 2-194 at week 8 (Females= 2.7 ± 0.4 mmol/l; Males =2.1 ± 0.2 mmol/l) and week 17 (Females= 3.0 ± 0.7 mmol/l; Males =1.8 ± 0.2 mmol/l). However, data were not collected in vehicle control at those time points; therefore, this difference is difficult to conclusively determine.

Total protein and globulin levels were significantly increased by 5-7% at week 6 and by 4-14% at week 15 in males and females exposed to 300 mg/kg BIA 2-093, 75 mg/kg BIA 2-195 and 250 mg/kg BIA 2-195. Similar increases were also observed in males and females exposed to 300 mg/kg BIA 2-194 and 300 mg/kg BIA 2-195 at weeks 8 and 17.

Urinalysis: All male dose groups exhibited consistently increased levels of protein in the urine at weeks 6 and 8 when compared to controls. At weeks 15 and 17, urinary protein levels were increased in males exposed to 300 mg/kg BIA 2-093, 300 mg/kg BIA 2-194 and 300 mg/kg BIA 2-195. Urinary volume was decreased by 50% in females exposed to 300 mg/kg BIA 2-194 and BIA 2-195 at week 8 only.

Organ weights: Absolute liver weights were increased in males and females exposed to 300 mg/kg BIA 2-093 (M= 37%; F=42%), 300 mg/kg BIA 2-194 (M= 41%; F=54%), 250 (M= 24%; F=24%) and 300 mg/kg (M= 27%; F=22%) BIA 2-195. Relative liver weights were increased in a similar manner in these dose groups.

Histopathology: Peer review: Yes; Adequate Battery: No. The following tissues were not examined: Harderian gland, lachrymal gland, nasal cavity/turbinates, and Zymbal gland.

The most common histopathological finding in this study was slight to moderate centrilobular hypertrophy in males and females from all groups except controls and rats exposed to 75 mg/kg BIA 2-195. The severity of this finding was less in the animals exposed to BIA 2-195 when compared to BIA 2-194 and BIA 2-093 and increased with dose. In addition, fatty hepatic vacuolation was observed to occur in animals from groups dosed with 300 mg/kg of their respective test article (1/6 animals from each dose group).

Kidney findings were more common and severe in males than in females in this study, an observation that is consistent with the increase in protein found in urine of male rats from this study. Hyaline droplets were observed in males from the high dose groups, but not females, in this study. This is a common toxicological finding in male rats, but was

not demonstrated by the Sponsor to result from increased binding to alpha-2-microglobulin. However, basophilic tubules were observed in males exposed to 300 mg/kg BIA 2-093, 300 mg/kg BIA 2-194 and BIA 2-195 and sporadically in females from the same dose groups (except for BIA 2-195).

Follicular hypertrophy of the thyroid, a common observation in rats exhibiting centrilobular hypertrophy, was observed with an increased incidence when compared to controls in males and females exposed to 300 mg/kg of the three test articles.

An increase in fundic dilated glands was observed in all female dose groups except for 75 mg/kg BIA 2-195. This finding was graded as minimal in all except one female in the BIA 2-093 dose group, suggesting that irritation due to dosing with test compounds may have occurred in the stomachs of female rats.

Organ	Finding	Males					Females				
		C	**2**	**3**	**5**	**6**	**C**	**2**	**3**	**5**	**6**
Kidney	**Hyaline Droplet**										
	Minimal	0	2	3	0	4	0	0	0	0	0
	Slight	0	4	3	0	0	0	0	0	0	0
	Basophilic Tubules										
	Minimal	0	4	2	2	2	0	0	1	0	0
Liver	**Centrilobular Hypertrophy**										
	Minimal	0	0	0	1	0	0	0	0	0	0
	Slight	0	2	2	4	4	0	2	3	0	3
	Moderate	0	4	4	1	2	0	3	3	0	1
Thyroid	**Follicular Epithelial Hypertrophy**										
	Minimal	0	2	1	1	2	1	2	2	1	2
	Slight	0	0	1	0	1	0	2	1	0	2
Stomach	**Diffuse Dilated Fundic Glands**										
	Minimal	0	0	0	0	0	0	2	3	2	4
	Slight	0	0	0	0	0	0	1	0	0	0

Reviewer's Table 6.3: Histopathology Findings (excerpted). Dose groups are defined in the Sponsor's Table provided in the "Doses" section of this study review. Data are the number of animals exhibiting this histopathological finding/ total number analyzed. C= Control, 2=Group 2- BIA 2-093, 3= Group 3- BIA 2-194 5= Group 5-BIA 2-195(250 mg/kg), 6=Group 6- BIA 2-195(300 mg/kg).

Toxicokinetics: TK parameters for BIA 2-194 and BIA 2-195 from male and female rats on Day 15 at 750 mg/kg/day and Weeks 6 and 15 at 300 mg/kg/day are provided in the Sponsor's Tables below. Minimal isomerization of BIA 2-195 to BIA 2-194 and BIA 2-194 to BIA 2-195 occurred in male or female rats at any time point in the study. Conversion of BIA 2-093 and BIA 2-194 to OXC, the major metabolite in rats, was extensive. The conversion of BIA 2-195 to OXC, in comparison, was not as robust.

Analyte: BIA 2-194									
Test / Comparator Substance	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24} (hr*ng/mL)	$t_{1/2}$ (hr)	Test / Comparator Substance	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24} (hr*ng/mL)	$t_{1/2}$ (hr)
Males, Day 15					Females, Day 15				
BIA 2-093	15500	0.500	63400	NC+	BIA 2-093	6180	0.500	55500	NC+
BIA 2-194	9620	2.00	72900	NC+	BIA 2-194	17900	0.500	98800	NC+
BIA 2-195	56.0	8.00	599	NC+	BIA 2-195	296	8.00	1390	NC+
Males, Week 6					Females, Week 6				
BIA 2-093	1760	0.500	18600	NC+	BIA 2-093	2390	12.0	34900	NC+
BIA 2-194	2160	0.500	25700	2.80	BIA 2-194	2440	2.00	27300	NC+
BIA 2-195	BLQ	NC+	NC+	NC+	BIA 2-195	133	0.500	1130	NC+
Males, Week 15					Females, Week 15				
BIA 2-093	2320	0.500	21300	3.43	BIA 2-093	2520	0.500	27300	6.77
BIA 2-194	4040	0.500	23500	4.72	BIA 2-194	6920	0.500	33200	NC+
BIA 2-195	37.5	8.00	150	NC+	BIA 2-195	118	8.00	800	NC+

NC+ Not calculated; a terminal monoexponential decline could not be unambiguously identified.
BLQ Below the lower limit of quantification (50 ng/mL).

Analyte: BIA 2-195									
Test / Comparator Substance	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24} (hr*ng/mL)	$t_{1/2}$ (hr)	Test / Comparator Substance	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24} (hr*ng/mL)	$t_{1/2}$ (hr)
Males, Day 15					Females, Day 15				
BIA 2-093	209	0.500	2440	NC+	BIA 2-093	166	2.00	2140	12.5
BIA 2-194	465	2.00	5920	NC+	BIA 2-194	648	0.500	8610	NC+
BIA 2-195	28500	0.500	324000	13.3	BIA 2-195	126000	2.00	1060000	24.6
Males, Week 6					Females, Week 6				
BIA 2-093	66.5	4.00	630	NC+	BIA 2-093	69.5	12.0	948	NC+
BIA 2-194	211	4.00	2730	11.1	BIA 2-194	191	8.00	2290	NC+
BIA 2-195	12100	0.500	172000	NC+	BIA 2-195	48200	0.500	439000	11.4
Males, Week 15					Females, Week 15				
BIA 2-093	87.0	12.0	892	NC+	BIA 2-093	35.5	12.0	494	NC+
BIA 2-194	278	0.500	2070	NC+	BIA 2-194	192	0.500	2080	NC+
BIA 2-195	14100	0.500	137000	8.14	BIA 2-195	34900	0.500	546000	NC+

NC+ Not calculated; a terminal monoexponential decline could not be unambiguously identified.

Sponsor's Tables: Toxicokinetics

Table 46 Toxicokinetic parameters of oxcarbazepine for male and female rats: Week 15

Males

Dose	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24} (hr*ng/mL)	λ_z (1/hr)	$t_{1/2}$ (hr)	R_{met}
300 mg/kg/day BIA 2-093	11000	0.500	138000	NC+	NC+	NC
300 mg/kg/day BIA 2-194	17500	0.500	145000	0.167	4.16	1.05
300 mg/kg/day BIA 2-195	3220	2.00	35900	NC+	NC+	0.259

Females

Dose	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24} (hr*ng/mL)	λ_z (1/hr)	$t_{1/2}$ (hr)	R_{met}
300 mg/kg/day BIA 2-093	13600	0.500	248000	NC+	NC+	NC
300 mg/kg/day BIA 2-194	16000	4.00	210000	NC+	NC+	0.846
300 mg/kg/day BIA 2-195	5290	4.00	76000	NC+	NC+	0.306

NC Not calculated.
NC+ Not calculated; a terminal monoexponential decline could not be unambiguously identified.

Sponsor's Table 46: Toxicokinetics

6.2.2. F. Study title: 13 Week Oral (Gavage) Dose Range Finding Study in the Rat

Key study findings:

- **Due to deaths in both males and females dosed with 1000 mg/kg, the high dose was lowered to 750 mg/kg.**
- **Salivation and distended abdomen were the most common clinical signs observed in all animals exposed to BIA 2-194. Vertically extended tail was common immediately after dosing in rats dosed with ≥ 500 mg/kg BIA 2-194 from Day 62 until the end of the study.**
- **Although there were hematological changes that suggested the possibility of reticulocytosis in male rats dosed with BIA 2-194, the Sponsor did not provide a measurement of reticulocyte number in this study.**
- **Blood cholesterol levels were increased in all females and in males exposed to ≥ 500 mg/kg BIA 2-194.**
- **Male rats exposed to 1000 mg/kg/day BIA 2-194 and females exposed to ≥ 500 mg/kg BIA 2-194 exhibited increased plasma ALP and bilirubin levels, suggestive of hepatic cholestasis. However, no histopathological evidence of cholestasis was observed.**
- **All male rat dose groups and females exposed to ≥ 500 mg/kg BIA 2-194 had elevated total plasma protein, albumin and globulin levels.**
- **Dark enlarged livers with increased relative and absolute weight were observed in all male and female dose groups. These abnormalities were coincident with increased incidence and severity of centrilobular hepatocyte hypertrophy.**
- **Absolute and relative kidney weights were increased in all male and female dose groups. However, a histopathological correlate was not found.**

- **Thyroid follicular epithelial hypertrophy was observed in all female dose groups and in male rats exposed to ≥ 500 mg/kg BIA 2-194.**
- **There was extensive conversion of BIA 2-194 to the major rat metabolite OXC.**

Study no.: 093-812
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 10/23/2002
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-194**,** Lots DL020611 & DL021009, Purity= 99.6 & 99.4%
Methods -

Doses: See table below; 0, 100 (LD), 100/500 (MD), 100/500/750 or 1000 (HD for males) 750 (HD for females) mg/kg
The final dose for each group was set after the dose range period of 2 weeks.

Group number	Number of animals		Dose level (mg/kg/day) BIA 2-194			
	Males	Females	Days 1 - 3	Days 4 - 7	Week 2	Week 3 onwards
1	10	10	0	0	0	0
2	10	10	100	100	100	100
3	10	10	100	500	500	500
4M	10	-	100	500	1000	1000
4F	-	10	100	500	1000	750
5	9	9	100	100	100	100
6	9	9	100	500	500	500
7M	9	-	100	500	1000	1000
7F	-	9	100	500	1000	750

Sponsor's Table

Species/strain: HsdBrlHan:WIST Wistar rats (b) (4)
Route: Oral gavage
Formulation/vehicle: 0.5% hydroxypropylmethylcellulose
Dosing solution analyses/drug stability and homogeneity: The Sponsor determined that storage of samples for analysis at 4°C may result in concentrations that are lower than the acceptable criteria for deviation from nominal concentration due to settling of the suspension. If stored frozen, the test article solutions were found to meet acceptance criteria.
Dose volume/infusion rate: 10 ml/kg

Satellite groups used for toxicokinetics or recovery: Groups 5-7 were used for TK
Age: 4-5 weeks
Weight: 181-238 g M; 126-165 g F

Observations times and results:

Mortality: HDF (5/19) and HDM (1/19) were euthanized *in extremis* during the period of time when they were receiving 1000 mg/kg/day (Days 9-11 and Day 44, respectively). The dose was then lowered in HDF to 750 mg/kg. Early deaths did not occur in any other dose group in the study.

Clinical signs: The most common clinical signs in this study were salivation and distended or firm abdomen. Hunched posture and "vertical tail" was observed in rats dosed with ≥ 500 mg/kg BIA 2-194. The onset of "vertical tail" was abrupt (on Day 62) and lasted until the end of the study. In addition to these clinical signs, rats dosed with 500 mg/kg also exhibited slight to moderate hypoactivity and unsteady gait which resolved by 3 hours post dose.

Body weights: Animals dosed with ≥ 500 mg/kg BIA 2-194 exhibited lower BW gain (7-27%) during the course of the study when compared to controls. Males and females exposed to 100 mg/kg/day exhibited similar BW gain to controls.

Food consumption: Food consumption was decreased slightly in HD males (7-9%) and females (9-24%) during weeks 3-6. There was no effect on food consumption in rats from the LD or MD groups during the study.

Ophthalmoscopy: No test article related abnormalities were observed.

Hematology: Hematological parameters were only assessed at one time point during this study. At week 13, a slight but statistically significant decrease in RBC count was observed in all male dose groups. Furthermore, MCV (6-7%) and MCH (6-8%) were slightly elevated in the MD and HD males. Since the Sponsor did not assess reticulocyte counts in these samples it is impossible to know if these alterations are occurring in concert with an increase in reticulocytes as previously demonstrated to occur in rats exposed to BIA 2-093, BIA 2-194 and BIA 2-195.

Clinical chemistry: The elevation in cholesterol in all female dose groups (24%, 48%, 41% in LD, MD and HD, respectively) and MD (47%) and HD (63%) males is consistent with observed changes in previous rat studies performed by the Sponsor. The increase in ALP in HD males (18%) and MD (31%) and HD (97%) females accompanied by the increase in plasma bilirubin in these groups suggests possible cholestasis. However, no histopathological evidence of cholestasis was observed in this study. Increased ALT was observed in all female dose groups (25%, 125%, 92% in LD, MD and HD, respectively) and in HD (21%) males. A decrease in AST was observed in all males and in HD females (17-32%), but the toxicological significance of this finding is unclear. As in previous rat studies where animals were exposed to BIA 2-194, a slight elevation in total plasma protein (7-13%) was observed in males and females.

Gross pathology: The only test article-related abnormality observed in BIA 2-194 exposed rats was a dose-dependent increase in abnormal size and color (darkened) of the liver, which was consistent with the organ weight and histopathology findings described below.

Organ weights: Absolute and relative liver weights were increased in all male and female dose groups in a dose-dependent manner. Absolute and relative kidney weights were increased in all male and female dose groups (Sponsor's Text Table #9).

Group/sex		Absolute and bodyweight related organ weights in comparison with Controls							
		1M	2M	3M	4M	1F	2F	3F	4F
Organ	Dose (mg/kg/day)	0	100	100/ 500	100/500 /1000	0	100	100/500	100/500 /1000/ 750
Liver	Abs (g)	11.37	**13.69** (+20%)**	**16.84** (+48%)***	**17.15** (+51%)***	7.51	**9.09** (+21%)***	**11.24** (+50%)***	**12.86** (+71%)***
	BWT (%)	3.13	**3.67** (+17%)***	**4.74** (+51%)***	**5.27** (+68%)***	3.45	**4.04** (+17%)***	**5.50** (+59%)***	**6.24** (+81%)***
Kidney	Abs (g)	2.01	**2.27** (+13%)*	**2.37** (+18%)**	**2.29** (+14%)*	1.41	1.53* (+9%)	**1.64** (+16%)***	**1.66** (+18%)***
	BWT (%)	0.56	**0.61** (+9%)*	**0.67** (+20%)***	**0.70** (+25%)***	0.65	0.68 (+5%)	**0.80** (+23%)***	**0.81** (+25%)***

* = p<0.05, ** = p<0.01, *** = p<0.001
Abs - Absolute
BWT - Bodyweight-related
Values considered to be notable are highlighted in bold.

Sponsor's Text Table 9 (excerpted): Organ weights

Histopathology: Peer review: Not documented; Adequate Battery: No. The following tissues were not analyzed: nasal cavity/ turbinates, Zymbal gland.

A dose-dependent increase in centrilobular hepatocyte hypertrophy incidence and severity was observed in all male and female dose groups. In females, an increase in thyroid follicular epithelial hypertrophy incidence was observed in all dose groups; the incidence in males was much lower. Finally, a dose-dependent increase in the number of corpora lutea was observed in female rats dosed with BIA 2-194.

Liver

		Males				**Females**			
Group		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Dose (mg/kg/day)		**0**	**100**	**100/500**	**100/500/ 1000**	**0**	**100**	**100/500**	**100/500/ 1000/750**
Centrilobular hepatocyte hypertrophy	Minimal	0	2	0	0	0	0	0	0
	Slight	0	4	2	0	0	7	0	0
	Moderate	0	0	8	7	0	0	9	7
	Total	**0**	**6 (60)**	**10 (100)**	**7 (100)**	**0**	**7 (70)**	**9 (100)**	**7 (100)**

Thyroid

		Males				Females			
Group		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Dose (mg/kg/day)		**0**	**100**	**100/500**	**100/500/ 1000**	**0**	**100**	**100/500**	**100/500/ 1000/750**
Follicular epithelial hypertrophy	Slight	1 (10)	3 (30)	3 (30)	1 (14)	1 (10)	6 (60)	8 (89)	7 (100)

Ovaries

		Females			
Group		**1**	**2**	**3**	**4**
Dose (mg/kg/day)		**0**	**100**	**100/500**	**100/500/ 1000/750**
Increased number of corpora lutea	**Total**	**1 (10)**	**1 (10)**	**2 (22)**	**6 (86)**
Prominent interstitial glands	**Total**	**1 (10)**	**1 (10)**	**4 (44)**	**4 (57)**

Sponsor's Text Tables 12-14: Histopathology Findings

Toxicokinetics: The results confirm extensive conversion of BIA 2-194 to OXC, the major metabolite in rat.

Text Table 10: Toxicokinetic parameters of BIA 2-005 for male and female rats following a single oral administration of BIA 2-194 at 100 mg/kg/day: Day 1 and following a repeated administration at 100 to 1000/750 mg/kg/day : Week 6

Males

Timepoint	Dose (mg/kg/day)	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-24h} (ng.h/mL)	λ_z (/h)	$t_{1/2}$ (h)	R_T
Day 1	100	2253	4.00	20605	0.242	2.87	1.00
Day 1	100	6260	2.00	51567	0.343*	2.02*	1.00
Day 1	100	2206	0.500	16141	0.183	3.79	1.01
	Dose (mg/kg/day)	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-24h} (ng.h/mL)	λ_z (/h)	$t_{1/2}$ (h)	R_O $
Week 6	100	2475	0.500	18448	0.127	5.46	0.895
Week 6	500	15109	2.00	131841	0.188*	3.70*	0.200
Week 6	1000	14965	4.00	213416	0.287*	2.42*	0.100

Females

Timepoint	Dose (mg/kg/day)	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-24h} (ng.h/mL)	λ_z (/h)	$t_{1/2}$ (h)	R_T
Day 1	100	5810	4.00	78677	NC	NC	NC
Day 1	100	3035	4.00	41427	NC	NC	NC
Day 1	100	2926	0.500	24761	0.0264#	26.3#	2.13
	Dose (mg/kg/day)	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-24h} (ng.h/mL)	λ_z (/h)	$t_{1/2}$ (h)	R_O $
Week 6	100	6428	4.00	52814	NC	NC	0.671
Week 6	500	8058	12.0	121239	NC	NC	0.585
Week 6	750	19192	0.500	151791	NC	NC	0.817

\# = Unreliable estimate; period over which λ_z was determined was less than twice the resultant $t_{½}$
* = Unreliable estimate; only 3 data points used in regression analysis
NC = Not calculated; terminal monoexponential phase could not be unambiguously identified
$ = R_o values were adjusted to account for changes in dose between Day 1 and Week 6

Sponsor's Text Table 10

Text Table 11: Toxicokinetic parameters of Oxcarbazepine for male and female rats following a single oral administration of BIA 2-194 at 100 mg/kg/day: Day 1 and following a repeated administration at 100 to 1000/750 mg/kg/day : Week 6

Males

Timepoint	Dose (mg/kg/day)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	λ_z (/h)	$t_{1/2}$ (h)	R_T
Day 1	100	9018	2.00	64208	0.162*	4.28*	1.02
Day 1	100	6608	2.00	60492	0.171*	4.05*	1.02
Day 1	100	9259	0.500	66636	NC	NC	NC
	Dose (mg/kg/day)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	λ_z (/h)	$t_{1/2}$ (h)	R_O $
Week 6	100	4318	2.00	34878	0.155	4.47	0.543
Week 6	500	NC*	NC*	NC*	NC*	NC*	NC*
Week 6	1000	20510	4.00	262890	0.220*	3.14*	0.395

Females

Timepoint	Dose (mg/kg/day)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	λ_z (/h)	$t_{1/2}$ (h)	R_T
Day 1	100	10761	2.00	99302	NC	NC	NC
Day 1	100	10483	2.00	92225	NC	NC	NC
Day 1	100	7704	8.00	85368	NC	NC	NC
	Dose (mg/kg/day)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	λ_z (/h)	$t_{1/2}$ (h)	R_O $
Week 6	100	4500	4.00	57351	0.162*	4.28*	0.578
Week 6	500	17913	8.00	240588	0.162*	4.28*	0.522
Week 6	750	17106	8.00	242138	NC	NC	0.378

* = Unreliable estimate; only 3 data points used in regression analysis
NC = Not calculated; terminal monoexponential phase could not be unambiguously identified
NC* = Not calculated; due to assay technical difficulties a concentration-time profile was not available
$ = R_o values were adjusted to account for changes in dose between Day 1 and Week 6

Sponsor's Text Table 11

6.2.2. G. Study title: 26 Week Oral (Gavage) Toxicity Study in the Rat

Key study findings:

- **A NOAEL could not be determined (<20 mg/kg) for this study due to the occurrence of chronic progressive nephropathy at a greater incidence and severity in all dose groups compared to vehicle controls. Polyuria, increased kidney weight, increased urinary electrolytes and increased urinary protein and leukocytes were also observed at all doses.**
- **Blood cholesterol was significantly elevated throughout the study in all female dose groups and in males exposed to 250 mg/kg.**
- **Total plasma protein and globulin were elevated in all female dose groups and males exposed to ≥ 75 mg/kg BIA 2-093.**
- **Centrilobular hypertrophy was observed in males and females dosed with ≥ 75 mg/kg BIA 2-093.**
- **Decreased absolute weight of the testes occurred in a dose-dependent manner in males. Coincident with this finding was complete degeneration of the germinal epithelium in 2/20 males dosed with 250 mg/kg. Testes from males exposed to lower doses were not subjected to histopathological analysis.**
- **Thyroid C cell hyperplasia (1/20) and follicular adenoma of the thyroid (1/20) was observed in two different males exposed to 250 mg/kg BIA 2-093.**
- **Distension of the uterus increased in incidence and severity in a dose-dependent manner in females.**
- **OXC was the major metabolite in rats dosed with BIA 2-093 for 26 weeks. However, the plasma levels ($AUC_{0\text{-}24hr}$) of OXC are approximately 3 to 5 fold lower than what was observed in the 4 week and 3 month rat study. The plasma levels of BIA 2-005 ($AUC_{0\text{-}24hr}$) are similar to those observed in the 4 week and 3 month rat studies. BIA 2-005 is produced in this study, in a dose proportional fashion where OXC production is less than dose proportional at 250 mg/kg.**

Study no.: 093-810
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 3/11/2002
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, Lot 0000012976, Purity= 99.6%
Methods -

Doses: 0, 20 (LD), 75 (MD), 250 (HD) mg/kg
Species/strain: Hsd:Sprague Dawley SD rats (b) (4)
Number/sex/group or time point: 20/dose/sex main study; 10 /dose/sex TK study
Route: Oral gavage
Formulation/vehicle: 0.5% hydroxypropylmethylcellulose
Dosing solution analyses/drug stability and homogeneity: All dose formulations were within ± 15% of the nominal concentration.
Dose volume/infusion rate: 5 ml/kg
Age: 4-5 weeks
Weight: 125-177 g M; 107-163 g F

Observations times and results:

Mortality: No BIA 2-093-related mortalities.

Clinical signs: Besides the observation of a firm abdomen in all HDF on day 40 and 41, hair loss was the only other consistent clinical sign observed in treated rats.

Body weights & Food consumption: There was no difference between control and treated groups in absolute BW, BW gain and food consumption.

Ophthalmoscopy: There were no abnormalities observed in the ophthalmoscopic evaluation.

Hematology: Although statistically significant changes in MCV, MCH and RBC were observed in HDM throughout the study, the magnitude of the change was between 2-4% of control and was, therefore, considered toxicologically irrelevant.

Clinical chemistry: Significant elevation of blood cholesterol in all female dose groups and in the HDMs was observed throughout the study. This finding has been observed in previous rat studies.

HDF exhibited a significant increase in ALP and ALT throughout the study. In addition, a decrease in AST was observed in the same dose groups that exhibited increased blood cholesterol. Although rats in previous studies have also demonstrated this decrease in AST, its toxicological significance is unknown.

Total plasma protein was increased in a dose-dependent manner in MDM, HDM and HDF and this increase was mainly due to an increase in plasma globulin at all time points. A decrease in blood urea was observed in HDF.

Group/sex		1M	2M	3M	4M	1F	2F	3F	4F
Dose level (mg/kg/day)		0	20	75	250	0	20	75	250
Parameter	Timepoint								
Cholesterol (mg/dL)	Week 8	108	+16*	+13*	+18**	108	+19**	+47***	+70***
	Week 13	105	+11	+10	+22**	108	+22**	+51***	+81***
	Week 26	130	+10	+22*	+37***	110	+25*	+57***	+75***
ALP (U/L)	Week 8	318	-4	-3	-9	210	-14	0	+45**
	Week 13	254	-2	-5	-4	161	-7	+6	+70**
	Week 26	215	-3	+2	0	164	-10	-5	+67***
ALT (U/L)	Week 8	44	+7	-2	-9	32	+3	+25*	+53***
	Week 13	43	-2	-14	-14	33	-12	+12	+30
	Week 26	52	-4	-15	-17	36	-17	+17	+36**
AST (U/L)	Week 8	72	+1	-7*	-12***	72	-8*	-8**	-22***
	Week 13	72	-6	-7	-14**	71	-10*	-8*	-23***
	Week 26	73	-5	-21**	-22**	76	-21***	-22***	-30***

Total protein (g/dL)	Week 8	6.8	-1	+1	+7***	6.7	+3**	+3***	+7***
	Week 13	6.9	-1	+4**	+9***	6.7	+3*	+4***	+7***
	Week 26	7.0	+3	+6**	+9***	6.9	+3	+6***	+9***
Albumin (g/dL)	Week 8	4.1	0	0	+2*	4.3	0	+2**	+5***
	Week 13	3.9	-3	0	+5***	4.2	0	+2	+2
	Week 26	3.7	0	+3	+5**	4.4	-2	+2	0
Globulin (g/dL)	Week 8	2.7	-4	+4	+15***	2.4	+8**	+4*	+8***
	Week 13	3.0	+3	+10**	+13***	2.5	+8*	+12**	+16***
	Week 26	3.3	+6	+9*	+12**	2.6	+4**	+12***	+19***
A/G ratio	Week 8	1.5	+7	0	-7*	1.8	-6	0	-6
	Week 13	1.3	0	-8**	-8*	1.7	-12*	-6*	-12**
	Week 26	1.1	0	0	0	1.7	-6	-6	-12**
Urea (mg/dL)	Week 8	40.2	-5	-1	+5	43.0	0	+8	-20**
	Week 13	40.5	-1	+1	0	44.9	+1	+1	-20***
	Week 26	35.2	-8	+2	-9	36.9	-1	-4	-2

* = p<0.05, ** = p<0.01, *** = p<0.001

Sponsor's Text Tables 1-4: Clinical Chemistry. Changes are presented as percent of control.

Urinalysis: Polyuria was observed in the HDM and HDF at week 26 and in MDF at week 13 (Reviewer's Tables 6.4 & 6.5). Elevated urinary electrolytes, in the form of increased levels of potassium and chloride, were also detected in the urine of HDM and HDF. In addition, leukocytes were detected in the urine of all females at week 13 and in HDF at week 26. Except for MDF at week 13, protein was elevated in the same groups.

Dose (mg/kg)	Volume (ml)	K (mmol/hr)	Cl (mmol/hr)	Na (mmol/hr)
0	2.1	0.027	0.009	0.003
20	1.6	0.024	0.008	0.003
75	1.9	0.025	0.009	0.004
250	3.7*	0.036*	0.017*	0.008*

*Reviewer's Table 6.4: Urinalysis at Week 26 of Male Rats Dosed with BIA 2-093. Electrolytes are presented as volume adjusted concentrations. * = p<0.05 when compared to control.*

A)

Dose (mg/kg)	Volume	Leukocytes	Protein	K (mmol/hr)	Cl (mmol/hr)
0	3	----	----	0.023	0.015
20	3.7	Increased	Increased	0.025	0.017
75	5.1*	Increased	----	0.028*	0.019
250	6.5*	Increased	Increased	0.032*	0.024*

B)

Dose (mg/kg)	Volume	Leukocytes	Protein	K (mmol/hr)	Cl (mmol/hr)
0	1.7	----	----	0.018	0.009
20	1.7	----	----	0.019	0.01
75	2.5	----	----	0.02	0.011
250	3.1	Increased	Increased	0.024	0.015*

Reviewer's Table 6.5: Urinalysis at A) Week 13 and B) Week 26 of Female Rats Dosed with BIA 2-093. Electrolytes are presented as volume adjusted concentrations.
** = p<0.05 when compared to control.*

Gross pathology: Accentuated hepatic lobular patterns were observed in MD (1/20) and HD (4/20) males. Abnormal size of submandibular lymph nodes was observed in all male dose groups (0/20, 3/20, 6/20, 5/20, in Control, LD, MD, and HD, respectively) and MDF (1/20) and HDF (4/20). Both observations occurred in a dose-dependent manner.

Organ weights: In all female dose groups and in MDM and HDM, absolute liver and kidney weights were increased. Gender specific changes in organ weights consisted of increased absolute and relative uterus weight in all female dose groups, decreased absolute testes weights in all male dose groups and increased absolute thyroid weights in HDMs.

Males

Group sex		Liver# g	Liver# %	Kidneys# g	Kidneys# %	Testes# g	Thyroid# glands g
1M	Mean	14.50	3.15	2.62	0.57	4.09	0.028
	S.D.	1.34	0.20	0.19	0.03	0.16	0.006
	N	16	16	17	17	17	17
2M	Mean	15.57	3.35*	2.72	0.59	3.89*	0.028
	S.D.	1.74	0.20	0.28	0.04	0.24	0.005
	N	20	20	20	20	20	20
3M	Mean	17.04***	3.73***	2.81**	0.62***	3.91*	0.030
	S.D.	1.85	0.21	0.23	0.03	0.32	0.007
	N	19	19	20	20	20	20
4M	Mean	20.41***	4.49***	2.89**	0.64***	3.84**	0.033*
	S.D.	2.78	0.36	0.39	0.04	0.25	0.007
	N	19	19	19	19	19	19

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Females

Group sex		Liver# g	Liver# %	Kidneys# g	Kidneys# %	Uterus# g	Uterus# %
1F	Mean	8.97	3.18	1.74	0.61	0.69	0.24
	S.D.	1.38	0.42	0.19	0.04	0.24	0.09
	N	18	18	20	20	20	20
2F	Mean	9.73*	3.33	1.86*	0.63	0.87*	0.30
	S.D.	1.31	0.33	0.15	0.04	0.42	0.15
	N	20	20	19	19	20	20
3F	Mean	11.19***	3.93***	1.90**	0.67***	0.90*	0.32*
	S.D.	1.62	0.39	0.17	0.04	0.39	0.14
	N	18	18	19	19	20	20
4F	Mean	13.34***	4.85***	2.00***	0.73***	1.26*	0.46*
	S.D.	1.09	0.32	0.19	0.06	1.37	0.51
	N	19	19	19	19	19	19

Sponsor's Table 18 (excerpted): Organ weights in male and female rats dosed with BIA 2-093. #= statistically analyzed; *=p<0.05, **=p<0.01, ***=p<0.001.

Histopathology: Peer review: Not documented; Adequate Battery: No. The following tissues were not analyzed: nasal cavity/ turbinates, Zymbal gland.

The incidence and severity of chronic progressive nephropathy increased in a dose-dependent manner in all male and female dose groups. Centrilobular hypertrophy was observed in MD and HD males and females. MDMs and HDMs also exhibited centrilobular hepatocyte vacuolation and one HDM exhibited focal hepatic necrosis. No hepatic abnormalities were observed in control males and females.

Decreased absolute weight of the testes was observed to occur in a dose-dependent manner in males. Coincident with this finding was minimal to slight degeneration of the germinal epithelium in 2/20 HDM. This was not observed in controls and histopathological analysis of LDM and MDM was not performed.

One HD male exhibited thyroid C cell hyperplasia while another exhibited follicular adenoma of the thyroid. Distension of the uterus increased in incidence and severity in a dose-dependent manner in females.

Observations	Group 1	Group 2	Group 3	Group 4
Male				
number of animals	20	20	20	20
Kidneys				
chronic progressive nephropathy				
~ minimal	14	9	6	6
~ slight	4	8	12	5
~ moderate	1	1	1	6
Liver				
centrilobular hepatocyte vacuolation				
~ minimal	0	0	4	4
~ slight	0	0	0	3
~ moderate	0	0	0	3
centrilobular hypertrophy				
~ slight	0	0	7	9
focal necrosis				
~ moderate	0	0	0	1
chronic inflammation				
~ moderate	0	0	0	1
Testes				
number examined	20	-	-	20
complete degeneration of germinal epithelium				
~ minimal	0	-	-	2
~ slight	0	-	-	1
Thyroid glands				
number examined	20	20	20	20
FOLLICULAR ADENOMA				
~ minimal	0	0	0	1
C cell hyperplasia				
~ moderate	0	0	0	1

Observations	Group 1	Group 2	Group 3	Group 4
Female				
number of animals	20	20	20	20

Kidneys				
chronic progressive nephropathy				
~ minimal	11	9	10	3
~ slight	5	7	7	14
~ moderate	0	2	2	3
Liver				
centrilobular hypertrophy				
~ slight	0	1	20	4
~ moderate	0	0	0	16
Uterus				
distended				
~ minimal	0	3	6	1
~ slight	2	3	6	4
~ moderate	2	3	1	3
~ marked	0	0	0	1

Sponsor's Table 20 (excerpted): Histopathology of Rats Dosed with BIA 2-093. Group 1= Control, Group 2= LD, Group 3= MD, Group 4= HD.

Toxicokinetics: Plasma concentrations of BIA 2-005 were consistently lower in males, but not in females, when assessed at week 26 and compared to day 1. OXC concentrations were consistently higher in each dose group and both sexes at week 26, when compared to day 1. However, when compared to similar doses in the 4 week (093-808) and 3 month (093-809) rat studies, the absolute plasma concentration of OXC was markedly lower (~3-5 fold) in male and female rats at week 26. Furthermore, the BIA 2-005 plasma concentration in rats from the 6 month study were similar to what was observed at the same doses in the 4 week and 3 month studies, suggesting that there was no difference in the total systemic exposure to BIA 2-005 in the rats in the 6 month study when compared to the rats in the 4 week and 3 month study. Although the Sponsor does not specifically address the inconsistency in the plasma concentration of OXC in the 6 month study, this finding suggests that either the metabolism of BIA 2-093 to OXC decreases with long-term, repeat dosing or an analytical or sample stability abnormality occurred in this study.

Metabolism of BIA 2-093 to OXC decreased between 75 and 250 mg/kg at Day 1 and increased in a less than dose-proportional manner at Week 26. At Week 26, there was a less than dose-proportional increase in C_{max} and $AUC_{0\text{-}24hr}$ in males and females when the dose was increased from 75 to 250 mg/kg.

BIA 2-005; Day 1

Males

Dose (mg/kg/day)	C_{max} (ng/ml)	t_{max} (h)	$AUC_{0\text{-}24h}$ (ng.h/ml)	λ_z (/h)	$t_{1/2}$ (h)	R_T
20	335	0.500	1577	NC	NC	NC
75	2354	0.500	5914	NC	NC	NC
250	3445	0.500	22408	0.136*	5.11*	1.04

Females

Dose (mg/kg/day)	C_{max} (ng/ml)	t_{max} (h)	$AUC_{0\text{-}24h}$ (ng.h/ml)	λ_z (/h)	$t_{1/2}$ (h)	R_T
20	447	0.500	2192	0.110*#	6.30*#	1.08
75	477	0.500	3901	0.183*	3.79*	1.01
250	2483	0.500	26323	0.0376*#	18.4*#	1.68

BIA 2-005; Week 26

Males

Dose (mg/kg/day)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	λ_z (/h)	$t_{1/2}$ (h)	R_O
20	354	0.500	980	NC	NC	0.621
75	1006	0.500	5877	NC	NC	0.994
250	2022	0.500	10990	NC	NC	0.490

Females

Dose (mg/kg/day)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	λ_z (/h)	$t_{1/2}$ (h)	R_O
20	650	0.500	3365	NC	NC	1.54
75	816	0.500	7648	NC	NC	1.96
250	2704	0.500	20525	0.0470#	14.7#	0.780

\# = Unreliable estimate; period over which λ_z was determined was less than twice the resultant $t_{½}$
* = Unreliable estimate; only 3 data points used in regression analysis
NC = Not calculated; terminal monoexponential phase could not be unambiguously identified

OXC; Day 1

Males

Dose (mg/kg/day)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	λ_z (/h)	$t_{1/2}$ (h)	R_T
20	310	2.00	1758	NC	NC	NC
75	773	4.00	3103	NC	NC	NC
250	204	8.00	2458	NC	NC	NC

Females

Dose (mg/kg/day)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	λ_z (/h)	$t_{1/2}$ (h)	R_T
20	470	2.00	4047	NC	NC	NC
75	844	0.500	8029	0.150*#	4.62*#	1.03
250	451	4.00	5517	0.103	6.72	1.09

OXC; Week 26

Males

Dose (mg/kg/day)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	λ_z (/h)	$t_{1/2}$ (h)	R_O
20	502	2.00	2236	NC	NC	1.27
75	1479	2.00	14425	NC	NC	4.65
250	3539	0.500	28388	NC	NC	11.5

Females

Dose (mg/kg/day)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-24h} (ng.h/ml)	λ_z (/h)	$t_{1/2}$ (h)	R_O
20	1936	12.0	19213	NC	NC	4.75
75	2405	0.500	22607	NC	NC	2.82
250	2487	8.00	28223	NC	NC	5.12

\# = Unreliable estimate; period over which λ_z was determined was less than twice the resultant $t_{½}$
* = Unreliable estimate; only 3 data points used in regression analysis
NC = Not calculated; terminal monoexponential phase could not be unambiguously identified

6.2.3. Rabbit

6.2.3. A. Study title: Oral Gavage Maximum Tolerated Dose Study in the Rabbit
Key study findings:

- **Food consumption and body weight were decreased in rabbits exposed to 320 mg/kg for 3 days. The 320-mg/kg dose was considered to be the MTD and was selected by the Sponsor as the HD for the pivotal embryo-fetal development study.**

Study no.: 093-814
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 1/2/2001
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, Lot 0000012976, Purity= 99.6%
Methods -

Doses: Pilot: 0, 40, 80, 160, 320 mg/kg (dose escalation study-3 days at each dose); Main study- 320 mg/kg (daily for 3 days)
Species/strain: New Zealand White rabbits
Number/sex/group or time point: 3 control and 3 doses in pilot; 3 dosed in main study
Route: Oral gavage
Formulation/vehicle: 0.5% hydroxypropylmethylcellulose
Dosing solution analyses/drug stability and homogeneity: Samples and reserve samples fell outside the specification limits, with 80-85% of the nominal concentration detected in the study samples.
Age: 4 months of age
Weight: 3-4 kg

Observations times and results:

Mortality & Clinical signs: No mortality or premature euthanasia occurred during this study. Loose feces was the only clinical sign observed.

Body weights & Food consumption: BW loss occurred in rabbits exposed to 320 mg/kg. In the pilot study, BW was reduced by 7% and in the main study was reduced by 5% after 6 days of dosing when compared to BW at baseline. There was also a marked reduction in food consumption (~56%) at 320 mg/kg in the pilot study. Food consumption was decreased by 49-60% between days 2-7 of dosing when compared to baseline in the main study.

Gross pathology: No dose related abnormalities were observed in rabbits exposed to BIA 2-093.

6.2.4. Dog

6.2.4. A. Study title: Oral Maximum Tolerated Dose (MTD) and 14 Day Repeat Dose Study in the Beagle Dog

Key study findings:

- **APTT was increased in dogs exposed to 160 mg/kg BIA 2-093.**
- **Urinary sodium, potassium and chloride excretion was decreased in dogs exposed to 7160 mg/kg BIA 2-093.**

Study no.: 093-815
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 3/12/1998
GLP compliance: Yes
QA statement: No
Drug, lot #, and % purity: BIA 2-093, BN980220, Purity = 100.2%
Methods -

Doses: Pilot: 20, 40, 80, 160 mg/kg; Main 160 mg/kg
Species/strain: Beagle dog
Number/sex/group or time point: 1/sex/group in pilot and main study
Route: Oral, capsule
Formulation/vehicle: Gelatin capsule
Dose volume/infusion rate: Once a day for 7 days (pilot) or 14 days (main)
Age: 6-8 months
Weight: 7-11 kg

Observations times and results:

Mortality and Clinical signs: No unscheduled deaths occurred during the study. In the pilot study, no clinical signs were observed at the 20 and 40 mg/kg dose. At the 80 mg/kg dose, the female dog vomited on Day 5 of dosing. On the last day of dosing at 160 mg/kg, the female vomited, had unsteady gait, tremors, and subdued behavior. These symptoms subsided 5.5 hours after dosing. In the 14 day study, loose and pale feces were observed during the first 8 days in the female and on day 14 in the male. No other clinical signs were observed during the 14 day study.

Body weights and Food consumption: BW loss (7-12%) was observed in the females in both studies, but not in the males. Most of the loss occurred during the 160 mg/kg dosing period. Consistent with this observation was the decrease in food consumption in the female in the pilot study during the HD phase. Food consumption was also decreased in the female, but not the male in the 14 day study.

ECG: No BIA 2-093 related effects were observed on heart rate.

Hematology: A dose-dependent increase in APTT was observed in both the male (18%) and female (43%) dogs in the pilot study and in the 14 day study in both animals (M= 22% increase; F= 34% increase). An increase in RBC, hemoglobin and hematocrit were

observed in the female exposed to 160 mg/kg for 14 days when compared to predose values.

Clinical chemistry: No effects were observed on clinical chemistry parameters.

Urinalysis: Urinary excretion of potassium and chloride was decreased in the male (80% and 65%, respectively) and female (41% and 85%, respectively) after exposure to 160 mg/kg BIA 2-093. Urinary sodium was decreased by 37% in the female, but not the male, exposed to 160 mg/kg BIA 2-093. This decrease in urinary sodium, potassium and chloride was also observed in the main study.

Gross pathology: The female in the pilot study had pale buccal mucus membranes. The lungs of the male in the 14-day study exhibited a few pale bilateral foci on the cardiac and diaphragmatic lobes. The female from this study exhibited several dark foci at the junction of the stomach and the duodenum.

6.2.4. B. Study title: Four Week Oral (Capsule) Repeat Dose Toxicity Study in the Beagle Dog

Key study findings:

- **BW gain was decreased in female dogs dosed with ≥ 20 mg/kg and in male dogs exposed to ≥ 80 mg/kg.**
- **A dose dependent increase in APTT was observed in dogs exposed to ≥ 20 mg/kg.**
- **Females exposed to 160 mg/kg exhibited a decrease in RBC, hematocrit, and hemoglobin.**
- **Females exposed to ≥ 80 mg/kg exhibited a dose-dependent decrease in absolute ovary and uterus weight.**
- **Low urinary levels of sodium, potassium and chloride were observed in 2 of 3 male dogs exposed to 160 mg/kg.**
- **Maximum $AUC_{0\text{-}24hr}$ of BIA 2-005 in dogs is approximately 2 fold below human steady state plasma levels achieved at the human maintenance dose of 800 mg.**

Study no.: 093-816
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 5/14/1998
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, BN980220, Purity= 100.2%
Methods -

Doses: 0, 20 (LD), 80 (MD), 160 (HD) mg/kg
Species/strain: Beagle dogs (b) (4)
Number/sex/group or time point: 3/sex/group
Route: Oral, capsule
Formulation/vehicle: Gelatin capsules
Dosing solution analyses/drug stability and homogeneity: Analysis of the capsules was not performed.

Age: 6-8 months old
Weight: 9-11 kg

Observations times and results:

Mortality: No unscheduled deaths were observed.

Clinical signs: Loose feces and vomiting were observed in all dose groups, including controls.

Body weights: Group mean body weight gain was decreased in a dose-dependent manner in all female dose groups (-0.06, -0.293, -0.607 kg, respectively). Group mean body weight gain was decreased in MDM and HDM (-0.297 and -0.323 kg, respectively). Absolute body weights were slightly decreased in a dose-dependent manner at the end of 5 weeks in males but not in females (M=11.3, 11.1, 11.0, 10.7; F= 10.3, 10.3, 10.7, 10.1; C, LD, MD, HD, repectively).

Food consumption: Food consumption was decreased in 2/3 HDM and 2/3 HDF during the first week of dosing. Consumption returned to normal during the remaining 3 weeks of dosing.

Ophthalmoscopy: No treatment-related opthalmoscopic findings were observed in this study.

ECG: No treatment-related alteration in heart rate was observed in dogs exposed to BIA 2-093. Waveforms were examined visually for gross abnormalities, but detailed analysis of ECG parameters was not performed.

Hematology: In the HDF, a 15% decrease in RBC count, hemoglobin and hematocrit was observed at the end of 4 weeks of dosing. This decrease may suggest a loss of RBCs due to hemolysis or slight suppression of bone marrow. However, no bone marrow abnormalities were observed in the histopathology assessment. A dose-dependent increase in APTT was observed in both males (11%, 21%, 51%, in LD, MD and HD, respectively) and females (12%, 33%, 58%, in LD, MD and HD, respectively) exposed to BIA 2-093 at the end of the 4 week dosing regimen.

Clinical chemistry: No drug-related effects on clinical chemistry analyses were observed in this study at the end of the 4 week dosing period.

Urinalysis: A dose-dependent decrease in urinary sodium levels was observed in all male dose groups (54%, 70%, 82%, in LD, MD, and HD, respectively). Urinary potassium and chloride were also decreased in the MDM (27% and 18%, respectively) and (32% and 35%, respectively) HDM. No further investigation of this finding was conducted in this study. Pre-study analysis of these parameters was not performed so it was impossible to determine if this observation existed before initiation of dosing.

Gross pathology: The pancreas of one HDM was reddened. Another HDM exhibited a dark foci (5x1mm) on the left intermediate lobe of the lung. This was not observed in the control group or in any of the females.

Organ weights: A dose-dependent decrease in the absolute weight of the ovaries and uterus in MDF (35% and 65%, respectively) and HDF (43% and 74%, respectively) was observed.

Histopathology: Peer review: Yes. Adequate Battery: No. Tissues not included are mammary tissue, seminal vesicle, cervix, fallopian tubes, lachrymal gland, and nasal cavity turbinates.

No consistent treatment-related effects were observed in this study. Specifically, no histopathogical findings were observed to correlate with the abnormal findings in the urinalysis of HDMs and organ weight findings in the uterus and ovary of BIA 2-093 exposed females.

Toxicokinetics: BIA 2-005 is the main metabolite of BIA 2-093 in dogs. Unlike in rodents, BIA 2-093 is detected in substantial amounts in plasma.

BIA 2-093

		Day1	Day 28	Day1	Day 28	Day1	Day 28	Day1	Day 28
Dose (mg/kg)	**Sex**	**C_{max} (ng/ml)**	**C_{max} (ng/ml)**	**t_{max} (h)**	**t_{max} (h)**	**$t_{1/2}$ (h)**	**$t_{1/2}$ (h)**	**AUC_{0-24hr} (ng*h/ml)**	**AUC_{0-24hr} (ng*h/ml)**
20	**M**	422	365	0.5	0.5	1.01	ND	1408	1679
80	**M**	1398	1149	2	2	ND	2.35	6162	2810
160	**M**	1623	1525	2	2	ND	2.27	11712	7528
20	**F**	619	625	2	2	0.927	ND	1485	ND
80	**F**	1405	540	2	2	1.54	ND	4500	3508
160	**F**	2382	2331	2	2	2.75	1.65	11489	13672

BIA 2-005

		Day1	Day 28	Day1	Day 28	Day1	Day 28	Day1	Day 28
Dose (mg/kg)	**Sex**	**C_{max} (ng/ml)**	**C_{max} (ng/ml)**	**t_{max} (h)**	**t_{max} (h)**	**$t_{1/2}$ (h)**	**$t_{1/2}$ (h)**	**AUC_{0-24hr} (ng*h/ml)**	**AUC_{0-24hr} (ng*h/ml)**
20	**M**	2350	2206	0.5	2	1.83	1.68	8794	9565
80	**M**	14351	13845	2	2	1.43	1.29	61302	42580
160	**M**	21774	26636	2	2	3.2	2.6	125282	99413
20	**F**	3545	3440	2	2	1.24	1.21	11062	9709
80	**F**	17545	8902	2	2	1.95	ND	68515	45918
160	**F**	30565	22098	2	2	2.7	1.4	160062	104447

OXC

Dose (mg/kg)	Sex	Day1 C_{max} (ng/ml)	Day 28 C_{max} (ng/ml)	Day1 t_{max} (h)	Day 28 t_{max} (h)	Day1 $t_{1/2}$ (h)	Day 28 $t_{1/2}$ (h)	Day1 AUC_{0-24hr} (ng*h/ml)	Day 28 AUC_{0-24hr} (ng*h/ml)
20	**M**	364	423	2	2	2.15	ND	1588	1722
80	**M**	2126	2113	2	2	3.4	1.97	12381	7963
160	**M**	2561	4429	4	2	ND	1.46	25287	22473
20	**F**	562	581	2	2	ND	ND	2350	1779
80	**F**	2357	1281	2	2	1.9	ND	12223	8000
160	**F**	3991	3249	4	2	ND	1.38	26742	19390

Reviewer's Table 6.6: Toxicokinetic Parameters of BIA 2-093, BIA 2-005 and OXC in Dogs Exposed to BIA 2-093 for 28 Days. ND= not determined

6.2.4. C. Study title: Three Month Oral (Capsule) Repeat Dose Toxicity Study in the Beagle Dog with a Four Week Recovery Period

Key study findings:

- **Dogs exposed to 160 mg/kg (HD) exhibited an infrequent pattern of symptoms such as rigidity, tremors and abnormal gait that the Sponsor states is suggestive of "seizure-like" activity. The NOEL for these observations is 80 mg/kg.**
- **APTT was increased in female dogs exposed to 160 mg/kg BIA 2-093. It is unclear if this effect is reversible due to the small group size (n=2) in the 4 week recovery group.**
- **Reversible increases in cholesterol and triglycerides were observed in HD males and females.**
- **Urinary excretion of sodium, potassium and chloride was decreased in HD females.**
- **Absolute and relative liver weight was increased in HD females.**
- **The mean steady state plasma concentration of BIA 2-005 in dogs at the NOEL for "seizure-like" clinical signs ($AUC_{0-24hrs}$= 35,877 ng*h/ml) is 9.3 fold lower than the steady state plasma concentration ($AUC_{0-24hrs}$= 336,147 ng*h/ml) in humans at the MRHD (1200 mg).**
- **The mean peak plasma concentration of BIA 2-005 in dogs at the NOEL for "seizure-like" clinical signs (Cmax = 8,716 ng/ml) is 2.6 fold lower than the mean peak plasma concentration (Cmax = 22,957 ng/ml) in humans at the MRHD (1200 mg).**

Study no.: 093-817
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 10/27/2000
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, Lot 0000012976, Purity= 99.6%
Methods-

Doses: 0, 20 (LD), 80 (MD), 160 (HD) mg/kg

Species/strain: Beagle dog (b) (4)
Number/sex/group or time point: 0 & 160 mg/kg: 3/sex/group. Recovery group consisted of an additional 2 control and 2 HD dogs/sex.
Route: Oral Capsule, single dose /day
Formulation/vehicle: Gelatin capsule
Age: 6-8 months old
Weight: 8.3-13.1 kg

Observations times and results:

Mortality: There were no unscheduled deaths during the study.

Clinical signs: HDMs exhibited unsteady gait (4/5), subdued (4/5) and lethargic (3/5) behavior, partially closed eyes (3/5), muscular rigidity (3/5), tremors (2/5) and temporary loss of limb function (1/5). HDFs exhibited unsteady gait (3/5), lethargic (2/5) and subdued (3/5) behavior, uncoordination (3/5), muscular rigidity (2/5), and limb rigidity (1/5). The Sponsor described these symptoms as seizure-like activity. These symptoms were not observed in dogs during the 4 week recovery period.

Body weights & Food consumption: BW gain in HDMs and HDFs was less than in controls and coincided with decreased food consumption. However, BW gain at the HD normalized during the later part of the study, resulting in no major difference in BW gain between groups at the end of the study.

Ophthalmoscopy: No drug-related effects were observed.

ECG: No drug-related effects on heart rate or ECG wave morphologies were observed.

Hematology: APTT was increased (12.3 sec in control vs. 21.1 sec in HD) in HDF, but not in males. This effect was not as pronounced in female dogs during the 4 week recovery period, but was still slightly elevated (individual values for APTT in female dogs after 4 weeks of recovery: 10.9 & 12.0 sec in control vs. 12.8 & 13.8 sec in HD).

Clinical chemistry: Cholesterol and triglyceride levels were increased in HDM (31% and 2.3 fold, respectively) and HDF (34% and 2 fold, respectively). This increase was not observed in dogs at the end of the 4 week recovery period.

Urinalysis: Although there was high variability, urinary sodium, potassium and chloride excretion was decreased in BIA 2-093 exposed animals was observed in HDM (31%, 16%, 28%, respectively) and HDF (35%, 24%, 33%, respectively) at the end of the three month dosing period. The decrease persisted in the 2 HDF to the end of the recovery period. However, the low number of individuals per group hinders a conclusive interpretation of the data.

Organ weights: Absolute liver weight was increased in male (19%, 11%,16% in LD, MD and HD, respectively) and female dogs (2%, 13%, 36% in LD, MD and HD, respectively) exposed to BIA 2-093, when compared to controls. The increase was not observed in animals in the recovery group.

Histopathology: Peer review: Yes; Adequate Battery: No. The following tissues were not included in the battery: lachrymal gland and nasal cavity turbinates.

Papillary urothelial hyperplasia (1/3 HD male) and pyelitis (1/3 MD, 1/3 HD female) was observed in the kidneys. Focal pneumonitis was observed in all female dose groups but not in female controls (1/3 LD, 1/3 MD, 2/3 HD) and in 1/3 control and 1/3 HD males. Finally, decreased serous secretion in the salivary glands was observed in 3/3 HDM and 1/3 HDF. No histopathological correlate for the increase in liver weights was reported by the Sponsor.

Toxicokinetics: BIA 2-005 was the main metabolite of BIA 2-093 in dogs. Unlike in rodents, BIA 2-093 was detected in substantial amounts in the plasma.

BIA 2-093

		Day1	Week 4	Week 13	Day1	Week 4	Week 13
Dose (mg/kg)	**Sex**	**Cmax (ng/ml)**	**Cmax (ng/ml)**	**Cmax (ng/ml)**	**AUC 0-24hr (ng*h/ml)**	**AUC 0-24hr (ng*h/ml)**	**AUC 0-24hr (ng*h/ml)**
20	**M**	598	293	237	2157	923	901
80	**M**	1683	564	161	5423	2064	1046
160	**M**	1709	1144	1055	9631	5077	3977
20	**F**	556	369	295	831	1096	784
80	**F**	1560	1297	890	5890	4089	3062
160	**F**	1671	1185	874	9391	4669	3855

BIA 2-005

		Day1	Week 4	Week 13	Day1	Week 4	Week 13
Dose (mg/kg)	**Sex**	**Cmax (ng/ml)**	**Cmax (ng/ml)**	**Cmax (ng/ml)**	**AUC 0-24hr (ng*h/ml)**	**AUC 0-24hr (ng*h/ml)**	**AUC 0-24hr (ng*h/ml)**
20	**M**	2831	1659	2104	11824	7119	9545
80	**M**	18339	13374	4827	72652	47956	27576
160	**M**	28333	26370	29004	186714	130578	116171
20	**F**	2842	2532	2333	8437	10949	8274
80	**F**	14457	12988	12605	55241	43406	44179
160	**F**	26504	25278	19205	176444	94228	99544

OXC

Dose (mg/kg)	Sex	Day1 Cmax (ng/ml)	Week 4 Cmax (ng/ml)	Week 13 Cmax (ng/ml)	Day1 AUC 0-24hr (ng*h/ml)	Week 4 AUC 0-24hr (ng*h/ml)	Week 13 AUC 0-24hr (ng*h/ml)
20	**M**	210	107	231	799	374	839
80	**M**	1238	719	566	7459	2571	3169
160	**M**	2319	1842	3051	18602	9048	13133
20	**F**	225	157	196	490	547	643
80	**F**	885	696	1143	3403	2372	3766
160	**F**	1780	1514	1768	16065	6391	9779

Reviewer's Table 6.7: Toxicokinetic Parameters of BIA 2-093, BIA 2-005 and OXC in Dogs Exposed to BIA 2-093 for 3 Months.

6.2.4. E. Study title: Six Month Oral (Capsule) Repeat Dose Toxicity Study in the Beagle Dog

Key study findings:

- **Vehicle, LD and MD groups were exposed to 40 and 80 mg/kg, respectively, for 8 months. HD was increased from 160 mg/kg to 210 mg/kg at day 50 and then continued for another 6 months.**
- **Clinical signs similar to the seizure-like activity observed in the three month study were observed in males exposed to 210 mg/kg/day BIA 2-093.**
- **APTT was increased in female dogs exposed to 160 and 210 mg/kg. The NOEL for this effect was 80 mg/kg.**
- **Agonal congestion and reddened lungs were observed in all female dose groups and in HD males, but the incidence was not dose-related.**

Study no.: 093-818
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 2/5/2002
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, Lot 0000012976, Purity = 99.6%
Methods -

Doses: 0, 40 (LD), 80 (MD), 160/210 (HD) mg/kg (160 was increased to 210 from Day 50-the end of the study).
Species/strain: Beagle Dog (b) (4)
Number/sex/group or time point: 4/sex/group
Route: Oral capsule, once a day for up to 33 weeks
Formulation/vehicle: Gelatin capsule
Age: 5-7 months
Weight: 5.8-8.6 kg

Observations times and results:

Mortality: There were no unscheduled deaths in this study.

Clinical signs: Vomiting was the most common clinical sign in all male and female groups, including controls. Females did not exhibit any of the clinical signs that were observed in previous studies after dosing with BIA 2-093, even when the HD was increased to 210 mg/kg. Male dogs, however, did exhibit the expected symptoms but only after the HD was increased from 160 to 210 mg/kg, on day 50. Beginning on the first day of the 210 mg/kg/ day dose, male HD dogs exhibited sporadic impaired mobility (2/4), unsteady gait (1/4), subdued demeanor (2/4), uncoordination (1/4), whole body muscular rigidity (1/4), labored breathing (1/4) and prostration (1/4). Also, beginning on Day 129 and lasting until the end of the study, one male dog in the HD group exhibited consistent hyperactivity (#405) and one exhibited sporadic hyperactivity (#403). These symptoms are similar to what the Sponsor characterized as seizure-like activity in Study 093-817.

Body weights: BW gain was suppressed in MDM (56%), HDM (55%), MDF (27%) and HDF (36%) between week 1 and week 34. Absolute weights at the end of the study were also decreased in a dose-dependent manner in males (6%, 8%, 9%, in LD, MD and HD, respectively) and females (14%, 20%, 20%, in LD, MD and HD, respectively), compared to controls.

Ophthalmoscopy: No line listings were provided for the ophthalmic examinations. The only evidence of ophthalmic examination is a one page letter from (b) (4) stating that no abnormalities existed.

ECG: With the exception of an abnormality observed in one HD male (#403) during week 12, no dose-related abnormalities were observed in heart rate or ECG trace morphology. In male #403, there was decreased T wave amplitude (0.1 mV vs. 0.6 mV, respectively) and the QRS complex (1.7 mV vs. 2.2mV, respectively) at 2 hours after dosing when compared to pre-dose. Since these changes were only observed in one dog and were not present when ECG analysis was performed on the same dog at 32 weeks, the findings are not considered test article-related.

Hematology: APTT was increased in HDFs at all weeks examined (Reviewer's Table 6.8). This increase in mean is due primarily to increases in 2 of the four females, which contributed to the increase in variability observed in this hematological parameter. A slight increase in APTT was also observed in LDFs and MDFs at week 13 and 21. A similar consistent increase in APTT was not observed in male beagles exposed to BIA 2-093. This increase in APTT is consistent with the results of previous studies (093-817 and 093-816).

APTT (sec)

Dose (mg/kg/day)	Week 0	Week 4	Week 5	Week 13	Week 21	Week 33
0	13.7 ± 0.6	13.7 ± 0.7	13.6 ± 0.5	13.0 ± 2.0	13.1 ± 1.7	12.3 ± 1.4
40	14.5 ± 0.7	18.1 ± 4.9	16.5 ± 3.3	25.7 ± 15.7	19.8 ± 7.4	27.8 ± 12.4
80	13.5 ± 1.9	13.5 ± 0.8	13.6 ± 0.9	17.0 ± 4.8	19.9 ± 6.8	16.8 ± 5.5
160	13.3 ± 0.9	29.6 ± 16.0	29.0 ± 15.4	-----	-----	-----
210	-----	-----	-----	55.3 ± 38.7	63.8 ± 48.8	45.2 ± 37.3

Reviewer's Table 6.8: APTT is Increased in Female Beagle Dogs Exposed to BIA 2-093. Data are mean +/- SD.

Clinical chemistry: Besides a slight increase in cholesterol in the HDFs at week 33 (4.2 +/- 0.2 mmol/L in control vs. 6.4 +/- 1.4 mmol/ L in HD), there were no treatment-related abnormalities in clinical chemistry parameters throughout the study.

Urinalysis: Due to marked variability among dose groups in the pre-study sample, it was impossible to determine if any alterations in urinalysis parameters were due to exposure to BIA 2-093.

Gross pathology: Pale foci in the heart were observed in 2/4 HDMs, but none of the females. In the lungs, the presence of pale foci were observed in 1/4 MDM and 1/4 MDF and 1/4 HDF. Reddened lungs were observed in 1/4 male controls, 2/4 HDM, 2/4 LDF, 3/4 MDF and 1/4 HDF. Reddening of the lung was not observed in female controls.

Organ weights: No treatment-related changes in organ weights were observed.

Histopathology: Peer review: Yes; Adequate Battery: No. The following tissues were not included in the battery: lachrymal gland and nasal cavity turbinates.

At necropsy, 2/4 HD males exhibited pale foci on the heart. Minimal to slight myocardial inflammatory cell infiltrate was observed in one of these animals upon histopathological analysis (#405). However, the histopathological finding was on the contralateral side of the lesion observed during necropsy and was, therefore, considered to be unrelated to the gross pathology finding. Agonal congestion was observed in 3/4 LD, 3/4 MD and 1/4 HD females and 1/4 male controls and 2/4 HD males. This finding was consistent with the observation of reddened lungs discovered during the necropsy of these dose groups. However, due to the lack of a dose-related increase in the incidence of these findings, the reddened lungs and agonal congestion were not considered to be drug related.

Toxicokinetics: BIA 2-005 was the main metabolite of BIA 2-093 in dogs. Unlike in rodents, BIA 2-093 was detected in the plasma.

BIA 2-093

Dose (mg/kg)	Sex	Day1 C_{max} (ng/ml)	Week 13 C_{max} (ng/ml)	Week 33 C_{max} (ng/ml)	Day1 AUC_{0-24hr} (ng*h/ml)	Week 13 AUC_{0-24hr} (ng*h/ml)	Week 33 AUC_{0-24hr} (ng*h/ml)
40	**M**	368	942	1491	990	1794	2017
80	**M**	454	994	1983	1372	1545	3320
160/ 210	**M**	1963	2052	3388	6893	6109	8005
40	**F**	1241	1164	1376	1781	1596	2223
80	**F**	2077	2403	1704	3090	4054	4582
160/ 210	**F**	1611	2185	3596	7385	7802	9006

BIA 2-005

Dose (mg/kg)	Sex	Day1 C_{max} (ng/ml)	Week 13 C_{max} (ng/ml)	Week 33 C_{max} (ng/ml)	Day1 AUC_{0-24hr} (ng*h/ml)	Week 13 AUC_{0-24hr} (ng*h/ml)	Week 33 AUC_{0-24hr} (ng*h/ml)
40	**M**	4789	8808	9709	20041	27771	28835
80	**M**	10072	14407	16891	37488	46399	45747
160/ 210	**M**	29867	46150	31561	126644	151343	124099
40	**F**	8285	11448	10181	24601	26028	24807
80	**F**	17532	25721	18516	56535	77497	62188
160/ 210	**F**	19579	40334	37657	117207	180225	133853

OXC

Dose (mg/kg)	Sex	Day1 C_{max} (ng/ml)	Week 13 C_{max} (ng/ml)	Week 33 C_{max} (ng/ml)	Day1 AUC_{0-24hr} (ng*h/ml)	Week 13 AUC_{0-24hr} (ng*h/ml)	Week 33 AUC_{0-24hr} (ng*h/ml)
40	**M**	248	645	1144	1802	2517	4295
80	**M**	956	1127	1838	4724	4209	6194
160/ 210	**M**	2784	2969	2884	14892	10765	13467
40	**F**	621	796	1405	1881	2078	4160
80	**F**	1671	1917	2353	5999	6692	9379
160/ 210	**F**	2195	3754	4782	13968	18364	17954

Reviewer's Table 6.9: Toxicokinetic Parameters of BIA 2-093, BIA 2-005 and OXC in Dogs Exposed to BIA 2-093 for 6 Months. The HD was increased from 160 to 210 mg/kg on Day 50. Therefore, week 13 and week 33 represent data from dogs exposed to 210 mg/kg.

6.2.4. D. Study title: Twelve Month Oral (Capsule) Repeat Dose Toxicity Study in the Beagle Dog

Key study findings:

- **One male and one female dog in the 160-mg/kg group were euthanized *in extremis*.**
- **Seizure-like clinical signs, similar to those observed in the 3 month and 6 month study, were observed in dogs dosed with 160 and 80 mg/kg BIA 2-093. NOEL was 40 mg/kg.**
- **One dog exposed to 160 mg/kg exhibited tremors on 60 of the 365 days of the study. This same dog also has extensive dilation of the brain ventricles.**
- **BW gain and food consumption were decreased in males and females exposed to 160 mg/kg BIA 2-093.**
- **APTT was elevated in all female dose groups at all time points and in male dogs dosed with 160 mg/kg on week 13 and week 26.**
- **Cholesterol levels were increased in a dose-dependent manner in females at all dose levels.**
- **A dose dependent increase in the severity of gallbladder epithelial vacuolation was observed in males dosed with ≥ 80mg/kg BIA 2-093.**

Study no.: 093-819
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 4/15/2003
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, Lot F0300001, Purity = 100%
Methods -

Doses: 0, 40 (LD), 80 (MD), 160 (HD) mg/kg/day. The HD group dose was changed several times during the first 3 weeks of the study before 160 mg/kg was chosen as the dose to be administered beginning on Day 21 until the end of the study.
Species/strain: Beagle dogs (b) (4)
Number/sex/group or time point: 4/sex/group
Route: Oral capsule
Formulation/vehicle: Gelatin capsule, once per day
Age: 5-7 months
Weight: 6.4-8.6 kg

Observations times and results:

Mortality: One male and one female HD dog (405M and 404F) were euthanized *in extremis* (day 16 and 99, respectively). When dosed at 210 mg/kg on Days 1 and 2, dog #405M exhibited subdued behavior, head shaking, abnormal/unsteady gait, muscular rigidity, lateral recumbence and unresponsive behavior. This animal was not dosed from days 3-5 due to these clinical signs. This animal was dosed at 160 mg/kg/day from days 7-13 and was returned to 210 mg/kg on day 14 at which point muscular rigidity, subdued behavior and lateral recumbence/unresponsive behavior was observed. On Day 16, animal 405M was euthanized *in extremis*. Animal 404F exhibited muscular rigidity,

lateral recumbence, abnormal and unsteady gait and subdued behavior beginning on Day 21. By the time of euthanasia *in extremis* on Day 99, animal 404F had experience several episodes that consisted of lateral recumbence, muscular rigidity, head shaking, tremors, subdued behavior and unsteady or abnormal gait.

Clinical signs: Vomiting, loose feces and salivation were the most common clinical symptoms in all dose groups, including the vehicle control. The HD and MD groups exhibited clinical signs that were observed in previous chronic studies in dogs dosed with BIA 2-093. During most of the study HDM and HDF exhibited episodic lateral recumbence (2/4 M; 1/4 F), muscular rigidity (4/4 M; 2/4 F), unresponsiveness (1/4 M), subdued behavior (4/4 M; 3/4 F), unsteady/ abnormal gait (4/4 M; 4/4 F), tremors (4/4 M; 2/4 F; Reviewer's Table 6.10). By day 72 in the study a "change in demeanor" which lasted until day 170 was documented in all HDM (4/4). A similar "change in demeanor" was observed in 3/4 HD females from Day 105-Day 167. Subdued behavior was observed in the MDM (3/4) and females (2/4) commencing on day 149 (M) and day 168 (F). Beginning on day 236 for MDMs and day 328 for MDFs, episodic tremors (2/4 M; 1/4 F) were also observed. The Sponsor has previously described these symptoms as seizure-like. Therefore from the clinical signs observed, the NOEL for the seizure-like symptoms is 40 mg/kg.

Animal Number	Sex	Dose (mg/kg)	# of Days Exhibiting Tremors
401	M	160	1
403	M	160	16
407	M	160	1
409	M	160	60
305	M	80	27
307	M	80	1
402	F	160	1
404	F	160	1
304	F	80	1

Reviewer's Table 6.10: Animals Exhibiting Tremors During the Course of the 12 Month Dog. In Animal # 40, severe ventricular dilation was detected necropsy.

Body weights: Mean BW gain was dramatically decreased in HDM (78%) and HDF (35%) over the course of 52 weeks. Absolute weights at the end of the study were decreased in a dose-dependent manner in treated male (4%, 8%, 17% in LD, MD and HD, respectively) and female (4%, 4%, 4%, 12% in LD, MD and HD, respectively) dogs, compared to controls.

Food consumption: Daily food consumption was variable, but generally lower in HDMs and HDFs than in controls.

Ophthalmoscopy: No line listings were provided for the ophthalmic examinations, only a one page letter from (b) (4) stating that no abnormalities existed.

ECG: Heart rates were assessed at week 51 of dosing. At this time, there were no discernable effects of the test article on heart rate or on the morphology of ECG waveforms.

Hematology: As was the case in shorter studies examining the effect of BIA 2-093 in dogs, APTT was elevated in female beagles in every dose group at every time point examined, when compared to control. Male dogs only exhibited an increase in APTT in the HD group at week 13 and week 26.

Male APTT (sec)

Dose (mg/kg/day)	Baseline	Week 13	Week 26	Week 52
0	12.4 ± 0.5	11.7 ± 0.7	12.4 ± 0.9	12.0 ± 0.5
40	13.4 ± 0.9	13.8 ± 1.1	14.2 ± 2.2	12.6 ± 1.1
80	13.7 ± 1.1	16.3 ± 4.4	16.4 ± 4.1	13.8 ± 2.7
160	13.4 ± 0.9	18.5 ± 4.7	25.3 ± 11.8	12.5 ± 1.2

Female APTT (sec)

Dose (mg/kg/day)	Baseline	Week 13	Week 26	Week 52
0	13.5 ± 0.4	17.8 ± 7.5	12.9 ± 0.4	12.0 ± 0.3
40	14.4 ± 0.5	37.3 ± 13.6	43.7 ± 18.4	16.6 ± 4.2
80	13.5 ± 0.7	18.9 ± 6.6	30.7 ± 19.0	24.4 ± 20.3
160	13.8 ± 1.1	58.7 ± 18.8	83.6 ± 29.1	41.2 ± 14.8

Reviewer's Table 6.11: APTT is Increased in Beagle Dogs Exposed to BIA 2-093. Data are mean +/- SD.

Clinical chemistry: Mean ALP was increased in HDF at week 52 when compared to vehicle controls (129 ± 31 U/L vs. 55 ± 17 U/L, respectively). However, the absolute value was not markedly different from the pre dosing analysis in the HDF (101 ±13 U/L). Cholesterol was also elevated in this group at 26 weeks (3.5 ± 0.4 mmol/L in controls and 5.7 ± 1.0 mmol/L in HDFs) and 52 weeks (3.7 ± 0.5 mmol/L in controls and 6.6 ± 2.4 mmol/L in HDFs). 2/4 LDF and 3/4 MDF also exhibited increased cholesterol levels during the course of the study suggesting a dose-dependent increase in cholesterol in females. This increase was also observed in males (2/4 MD, 1/4 HD), but was not as robust as in the females.

Urinalysis: No consistent dose-related changes were observed in urinalysis parameters.

Gross pathology: One HD male (#409) exhibited extensive dilation of the cerebral ventricles and hydrocephalus. This animal exhibited the greatest number of days experiencing tremor with 58/60 of the days with tremor occurring at day 220 or later. The coincidence of excessive tremors and ventricular dilation in this animal suggests a potential relationship. There were no other findings that occurred in a dose-dependent manner.

Organ weights: Absolute liver weights were increased in a dose-dependent manner in females (12%, 26%, 31%, LD, MD, HD, respectively), but not in males. A similar increase in relative liver weight was observed. Absolute, but not relative, kidney (24%) and testes (21%) weights were decreased in HDM.

Histopathology: Peer review: Yes; Adequate Battery: No. The following tissues were not included in the battery: lachrymal gland and nasal cavity turbinates.

Animal 409 which exhibited extensive ventricular dilation in the brain also exhibited focal perivascular lymphocytic cuffing and epithelial vacuolation in the choroid plexus. Perivascular lymphocytic cuffing also occurred in one MDM and one MDF and not in control animals of either gender.

A dose-dependent increase in the severity of gallbladder epithelial vacuolation was observed in MDM (3/4 slight) and HDM (1/4 slight, 1/4 moderate). This was also seen in one LDF (1/4 slight) and one HDF (1/4 minimal, 1/4 slight, 1/4 moderate). Slight macrophage infiltration accompanied the increase in epithelial vacuolation.

Toxicokinetics: BIA 2-005 was the main metabolite of BIA 2-093 in dogs. Unlike in rodents, BIA 2-093 was detected in the plasma.

BIA 2-093

		Day1	Week 26	Week 52	Day1	Week 26	Week 52
Dose (mg/kg)	**Sex**	**Cmax (ng/ml)**	**Cmax (ng/ml)**	**Cmax (ng/ml)**	**AUC 0-24hr (ng*h/ml)**	**AUC 0-24hr (ng*h/ml)**	**AUC 0-24hr (ng*h/ml)**
40	**M**	1975	2341	1121	2389	2977	1729
80	**M**	3024	1062	2595	4589	2641	4540
210/160	**M**	1588	2807	1117	6769	5877	2524
40	**F**	723	1316	940	1799	1966	1440
80	**F**	2958	3067	2172	4841	3588	2955
210/160	**F**	4054	2341	1407	8052	8612	4331

BIA 2-005

		Day1	Week 26	Week 52	Day1	Week 26	Week 52
Dose (mg/kg)	**Sex**	**Cmax (ng/ml)**	**Cmax (ng/ml)**	**Cmax (ng/ml)**	**AUC 0-24hr (ng*h/ml)**	**AUC 0-24hr (ng*h/ml)**	**AUC 0-24hr (ng*h/ml)**
40	**M**	26363	12398	10895	62570	40276	33400
80	**M**	42191	17742	27299	121450	70539	74031
210/160	**M**	28179	24913	21139	170751	108163	84022
40	**F**	16638	15465	15337	39262	41213	34126
80	**F**	21197	27445	34323	78043	71548	77711
210/160	**F**	454414	31897	28349	150996	146599	121475

OXC

Dose (mg/kg)	Sex	Day1 Cmax (ng/ml)	Week 26 Cmax (ng/ml)	Week 52 Cmax (ng/ml)	Day1 AUC 0-24hr (ng*h/ml)	Week 26 AUC 0-24hr (ng*h/ml)	Week 52 AUC 0-24hr (ng*h/ml)
40	**M**	1933	1831	1266	8074	7523	4862
80	**M**	3768	2949	2718	15768	12057	9352
210/160	**M**	3036	2761	2559	22006	14738	11339
40	**F**	1696	2377	1674	5780	8028	5211
80	**F**	2863	3767	3812	12847	11500	11432
210/160	**F**	3910	3867	4031	20186	17157	18288

Reviewer's Table 6.12: Toxicokinetic Parameters of BIA 2-093, BIA 2-005 and OXC in Dogs Exposed to BIA 2-093 for 12 Months.

6.3 Immunotoxicity Studies:

Reviewer's Summary:

- **The metabolites of BIA 2-093 (BIA 2-194, BIA 2-195 and OXC) were not contact sensitizers in the mouse LLNA.**

6.3.1 Mouse Local Lymph Node Assay (LLNA):
6.3.1. A. Study Title: BIA 2-194: Local Lymph Node Assay in the Mouse.

Key study findings:

- **BIA 2-194 was not a contact sensitizer in the murine LLNA assay.**

Study no.: 093-868
Study report location: EDR
Conducting laboratory and location: (b) (4).
Date of study initiation: 9/2/08
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: BIA 2-194; Lot 2070-2-1, Purity= 100%
Methods:

Doses: 10 % (LD), 25% (MD), 50% (HD) (w/w) in DMSO
Species/strain: Female CBA/Ca (CBA/CaOlaHsd) mice, (b) (4)
Number/sex/group: 4 females/ dose group
Route: Application to the dorsal surface of each ear
Dosing solution analyses/drug stability: Not examined in this study
Dose volume/infusion rate: 25 µl/ear/ day for 3 days
Age/ Weight: 8-12 wks; 15-23 g

Observations times and results:

Mortality: Mortality was not observed during this study at any dose.

Clinical signs: No manifestation of abnormal clinical signs occurred. At the highest dose in the preliminary study (50% w/w), test article precipitate was observed on the ears of the test animals suggesting that this was the MFD for this study.

Body weights: All animals in the study gained weight during the dosing period. No dose-dependent decrease in total BW or BW gain was observed.

Proliferative Response: The Stimulation Index ((dpm/node in BIA 2-194 dosed)/ (dpm/node in vehicle control)) did not exceed the threshold to be considered a sensitizer (SI>3) in any dose group (1.8, 2.1, 1.6; LD, MD, HD respectively). Therefore, BIA 2-194 did not exhibit sensitizing activity in the LLNA. The positive control, α-hexylcinnamaldehyde, did exhibit sensitizing activity in the LLNA (SI >3).

6.3.1. B. Study Title: BIA 2-195: Local Lymph Node Assay in the Mouse.

Key study findings:

- **BIA 2-195 was not a contact sensitizer in the murine LLNA assay.**

Study no.: 093-869
Study report location: EDR
Conducting laboratory and location: (b) (4).
Date of study initiation: 9/2/08
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: BIA 2-195, Lot PC040414, Purity= 100%
Methods:

Doses: 10 % (LD), 25% (MD), 50% (HD) (w/w) in DMSO
Species/strain: Female CBA/Ca (CBA/CaOlaHsd) mice, (b) (4)
Number/sex/group: 4 females/ dose group
Route: Application to the dorsal surface of each ear
Dosing solution analyses/drug stability: Not examined in this study
Dose volume/infusion rate: 25 µl/ear/ day for 3 days
Age/ Weight: 8-12 wks; 15-23 g

Observations times and results:

Mortality: Mortality was not observed during this study at any dose.

Clinical signs: No manifestation of abnormal clinical signs occurred. At the highest dose in the preliminary study (50% w/w), test article precipitate was observed on the ears of the test animals suggesting that this was the MFD for this study.

Body weights: No dose-dependent change in total BW or BW gain was observed.

Proliferative Response: The Stimulation Index did not exceed the threshold to be considered a sensitizer (SI>3) in any dose group (2.5, 1.5, 2.5; LD, MD, HD respectively). Therefore, BIA 2-195 did not exhibit sensitizing activity in the LLNA. The

positive control, α-hexylcinnamaldehyde, did exhibit sensitizing activity in the LLNA (SI >3).

6.3.1. C. Study Title: Oxcarbazepine: Local Lymph Node Assay in the Mouse

Key study findings:

- **The Stimulation Index was increased in a dose dependent manner by OXC. However, an SI >3 was not observed in any of the doses tested in this Study (up to 25%). Therefore, OXC was not a contact sensitizer under the conditions of this study.**

Study no.: 093-870
Study report location: EDR
Conducting laboratory and location: (b) (4).
Date of study initiation: 9/2/08
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: OXC, Lot FO40003, Purity= 100%
Methods:

Doses: 5 % (LD), 10% (MD), 25% (HD) (w/w) in DMSO
Species/strain: Female CBA/Ca (CBA/CaOlaHsd) mice, (b) (4)
Number/sex/group: 4 females/ dose group
Route: Application to the dorsal surface of each ear
Dosing solution analyses/drug stability: Not examined in this study
Dose volume/infusion rate: 25 µl/ear/ day for 3 days
Age/ Weight: 8-12 wks; 15-23 g

Observations times and results:

Mortality: Besides the animal that was euthanized *in extremis* during the preliminary study, no mortality was observed.

Clinical signs: In the preliminary study, the mouse exposed to 50% OXC was euthanized *in extremis* on Day 1 mainly due to the exhibition of hunched posture, lethargy, ptosis and splayed gait. Therefore, the highest dose in the main study was chosen to be 25%. No overt clinical signs were observed in any dose group of the main study.

Body weights: No dose-dependent change in total BW or BW gain was observed.

Proliferative Response: A dose-dependent increase in Stimulation Index (1.4, 1.8, 2.2; LD, MD, HD) was observed. However, none of these values exceeded the threshold of >3 considered to be the hallmark of sensitizers. Therefore, OXC did not exhibit sensitizing activity at the doses used in this study. It is possible that an SI >3 could be obtained at higher doses of OXC than 25%.

7. Genetic Toxicology

Reviewer's Summary:

- **Since the Sponsor did not measure the metabolite profile of BIA 2-093 in the presence and absence of rat hepatic S9 fraction, it is unclear if adequate concentrations of BIA 2-194, BIA 2-195 and OXC were present in the *in vitro* genotoxicity assays.**
- **BIA 2-093, BIA 2-194, BIA 2-195, (b) (4) and OXC were not mutagens in the bacterial reverse mutation assay.**
- **BIA 2-093 was a weak mutagen in the *in vitro* mouse lymphoma assay.**
- **BIA 2-093 was a clastogen that caused chromatid deletion in the CHO cell assay, but not in the human peripheral blood lymphocyte assay.**
- **BIA 2-093 was negative in the *in vivo* mouse micronucleus assay and the unscheduled DNA synthesis assay.**

7.1. *In vitro* reverse mutation assay in bacterial cells (Ames)

7.1.1. Study title: Bacterial Reverse Mutation Test

Key study findings:

- **Purity data and a certificate of analysis were not provided for test article.**
- **BIA 2-093 was negative, as tested, in the bacterial reverse mutation assay. However it is unknown if the entire profile of BIA 2-093 metabolites was represented in this assay.**

Study no.: 093-820
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 6/11/1997
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, Lot #23671 (not found in batch analysis document in 3.2.S.4.4), Purity: Not Provided
Methods
Strains:
S. *typhimurium* TAl535 his G46 *rfa Δ*uvrB.
S. typhimurium TAl537 his C3076 *rfa Δ*uvrB.
S. typhimurium TA98 his D3052 *rfa Δ*uvrB- pKM101
S. *typhimurium* TA100 his G46 *rfa Δ*uvrB- pKM101
E. coli WP2uvrA trpE *rfa' Δ*uvrA

Concentrations used in definitive study: 5000, 1000, 200, 40, 8 and 0 µg/plate with and without S-9.

Basis of concentration selection: To determine cytotoxicity, the following concentrations of BIA 2-093 were tested: 5000, 1000, 200, 40, 8, 1.6 and 0 µg/plate. After 24 hours, growth was observed up to 5000 µg/ plate with the rat liver S9 fraction but restricted growth was observed at

the highest dose in the absence of the S9 fraction in TA 98. The Sponsor did not provide data regarding the magnitude of the restricted growth.

Negative controls: 10% DMSO

Positive controls:
Without S-9:
TAl535- Sodium azide (SA) l µg/plate
TAl537- 9-Aminoacridine (9AA) 50 µg/plate
TA98- Nitrofluorene (2NF) 0.5 µg/plate
TA100- Sodium azide (SA) 1 µg/plate
WP2 uvrA- 4-nitroquinoline-N-oxide (NQO) l µg/plate

With S-9: All strains exposed to 2-Aminoanthracene (2AA) 2µg/plate. The Sponsor states that the S-9 fraction was tested for sterility, metabolic activity and protein content before use. Data from these analyses were not provided in the study report.

Incubation and sampling times: Both the plate incorporation and the pre-incubation methods were employed. In the plate incorporation method, plates were incubated at 37^{o}C for 66 hours after the hardening of the agar. For the pre-incubation method, the pre-incubation period lasted for 20 minutes at 37^{o}C. After addition of the pre-incubated mixture to Petri dishes, the dishes were incubated at 37^{o}C for 66 hours.

Study validity: The results for the negative and positive controls for each strain were within the range of historic controls; therefore, the study is valid. The concentrations of BIA 2-093 employed are consistent with recommendations of regulatory guidances.

Results: No concentration-dependent increase in the number of revertants was observed in cultures exposed to BIA 2-093 by either the plate incorporation or pre-incubation method. The results of these studies suggest that BIA 2-093 is not a mutagen in the bacterial reverse mutation assay. However, since BIA 2-093 is essentially a pro-drug for BIA 2-194, the active metabolite, it is important to understand the genotoxic potential of this metabolite. Since the Sponsor did not expressly measure BIA 2-194 in this assay, it is unknown if adequate levels of BIA 2-194 required to assess its genotoxic potential were present in the assay.

7.1.2 Study title: **BIA 2-194: Testing for Mutagenic Activity with Salmonella typhimurium TA 1535, TA 100, TA 1537 and TA 98 and Escherichia coli WP2uvrA**

Key study findings:

- **BIA 2-194 is not mutagenic in the bacterial reverse mutation assay.**

Study no.: 093-821
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 5/11/2006
GLP compliance: Yes

QA statement: Yes
Drug, lot #, and % purity: BIA 2-194, F0300001/2070-2-1 (Bial), Purity = 99.6%

Methods
Strains:
S. *typhimurium* TAl535 his G46 *rfa Δ*uvrB.
S. typhimurium TAl537 his C3076 *rfa Δ*uvrB.
S. typhimurium TA98 his D3052 *rfa Δ*uvrB- pKM101
S. *typhimurium* TA100 his G46 *rfa Δ*uvrB- pKM101
E. coli WP2uvrA trpE *rfa' Δ*uvrA

Concentrations used in definitive study: 5000, 1667, 500, 167, 50, 17 and 0 µg/plate

Basis of concentration selection: To determine toxicity, the following concentrations of BIA 2-194 were tested with and without rat liver S9: 5000, 1667, 500, 167, 50, 17 and 0 µg/plate. No concentration-dependent toxicity or precipitation was observed after incubation.

Negative controls: 10% DMSO

Positive controls:
Without S-9:
TAl535- Sodium azide (SA) l µg/plate
TAl537- 9-Aminoacridine (9AA) 80 µg/plate
TA98- Nitrofluorene (2NF) 1 µg/plate
TA100- Sodium azide (SA) 1 µg/plate
WP2 uvrA- N-Ethyl-N-Nitro-N-nitrosoguanidine (ENNG) 2 µg/plate

With S-9:
All strains exposed to 2-Aminoanthracene (2AA) 0.5-20 µg/plate. The activity of the S9 fraction was confirmed with 2-AAF, 4-AAF, benzo(a)pyrene and dimethylaminoazobenzene.

Incubation and sampling times: Both the plate incorporation and the pre-incubation methods were employed. In the plate incorporation method, plates were incubated at 37^{o}C for 48-72 hours after the hardening of the agar. For the pre-incubation method, the pre-incubation period lasted for 20 minutes at 37^{o}C. After addition of the pre-incubated mixture to Petri dishes, the dishes were incubated at 37^{o}C for 48-72 hours.

Study validity: The results for the negative and positive controls for each strain were within the range of historic controls; therefore, the study is valid. The concentrations of BIA 2-194 employed are consistent with recommendations of regulatory guidances.

Results: No concentration-dependent increase in the number of revertants was observed in cultures exposed to BIA 2-194 by either the plate incorporation or pre-incubation method. Therefore, BIA 2-194 was not mutagenic in the bacterial reverse mutation assay.

7.1.3 Study title: **BIA 2-195: Testing for Mutagenic Activity with Salmonella typhimurium TA 1535, TA 100, TA 1537 and TA 98 and Escherichia coli WP2uvrA**

Key study findings:

- **BIA 2-195 was not mutagenic in the bacterial reverse mutation assay.**

Study no.: 093-822
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 5/11/2006
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-195, PC041110, Purity= 100%

Methods
Strains:
S. *typhimurium* TAl535 his G46 *rfa Δ*uvrB.
S. typhimurium TAl537 his C3076 *rfa Δ*uvrB.
S. typhimurium TA98 his D3052 *rfa Δ*uvrB- pKM101
S. *typhimurium* TA100 his G46 *rfa Δ*uvrB- pKM101
E. coli WP2uvrA trpE *rfa' Δ*uvrA

Concentrations used in definitive study: 5000, 1667, 500, 167, 50, 17 and 0 µg/plate

Basis of concentration selection: To determine toxicity, the following concentrations of BIA 2-195 were tested with and without rat liver S9: 5000, 1667, 500, 167, 50, 17 and 0 µg/plate. No concentration-dependent toxicity or precipitation was observed.

Negative controls: 10% DMSO

Positive controls:
Without S-9:
TAl535- Sodium azide (SA) l µg/plate
TAl537- 9-Aminoacridine (9AA) 80 µg/plate
TA98- Nitrofluorene (2NF) 1 µg/plate
TA100- Sodium azide (SA) 1 µg/plate
WP2 uvrA- N-Ethyl-N-Nitro-N-nitrosoguanidine (ENNG) 2 µg/plate

With S-9:
All strains exposed to 2-Aminoanthracene (2AA) 0.5-20 µg/plate. The activity of the S9 fraction was confirmed with 2-AAF, 4-AAF, benzo(a)pyrene and dimethylaminoazobenzene.

Incubation and sampling times: Both the plate incorporation and the pre-incubation methods were employed. In the plate incorporation method, plates were incubated at 37^{o}C for 48-72 hours after the hardening of the agar. For the pre-incubation method, the pre-incubation period lasted

for 20 minutes at 37°C. After addition of the pre-incubated mixture to Petri dishes, the dishes were incubated at 37°C for 48-72 hours.

Study validity: The results for the negative and positive controls for each strain were within the range of historic controls; therefore, the study is valid. The concentrations of BIA 2-195 employed are consistent with recommendations of regulatory guidances.

Results: No concentration-dependent increase in the number of revertants was observed in cultures exposed to BIA 2-195 by either the plate incorporation or pre-incubation method. These results suggest that BIA 2-195 was not mutagenic in the bacterial reverse mutation assay.

7.1.4 Study title: **Oxcarbazepine: Testing for Mutagenic Activity with Salmonella typhimurium TA 1535, TA 100, TA 1537 and TA 98 and Escherichia coli WP2uvrA**

Key study findings:

- **OXC was not mutagenic in the bacterial reverse mutation assay.**

Study no.: 093-823
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 5/11/2006
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: OXC, F040003, Purity = 99.4%

Methods
Strains:
S. *typhimurium* TAl535 his G46 *rfa Δ*uvrB.
S. typhimurium TAl537 his C3076 *rfa Δ*uvrB.
S. typhimurium TA98 his D3052 *rfa Δ*uvrB- pKM101
S. *typhimurium* TA100 his G46 *rfa Δ*uvrB- pKM101
E. coli WP2uvrA trpE *rfa' Δ*uvrA

Concentrations used in definitive study: 5000, 1667, 500, 167, 50, 17 and 0 µg/plate

Basis of concentration selection: To determine toxicity, the following concentrations of OXC were tested with and without rat hepatic S9 fraction: 5000, 1667, 500, 167, 50, 17 and 0 µg/plate. No concentration-dependent toxicity was observed. Precipitation was observed at 5000 µg/plate with and without the S9 fraction.

Negative controls: 10% DMSO

Positive controls:
Without S-9:
TAl535- Sodium azide (SA) l µg/plate
TAl537- 9-Aminoacridine (9AA) 80 µg/plate

TA98- Nitrofluorene (2NF) 1 µg/plate
TA100- Sodium azide (SA) 1 µg/plate
WP2 uvrA- N-Ethyl-N-Nitro-N-nitrosoguanidine (ENNG) 2 µg/plate

With S-9:
All strains exposed to 2-Aminoanthracene (2AA) 0.5-20 µg/plate. The activity of the S9 fraction was confirmed with 2-AAF, 4-AAF, benzo(a)pyrene and dimethylaminoazobenzene.

Incubation and sampling times: Both the plate incorporation and the pre-incubation methods were employed. In the plate incorporation method, plates were incubated at 37°C for 48-72 hours after the hardening of the agar. For the pre-incubation method, the pre-incubation period lasted for 20 minutes at 37°C. After addition of the pre-incubated mixture to Petri dishes, the dishes were incubated at 37°C for 48-72 hours.

Study validity: The results for the negative and positive controls for each strain were within the range of historic controls; therefore, the study is valid. The concentrations of OXC employed are consistent with recommendations of regulatory guidances.

Results: No concentration-dependent increase in the number of revertants was observed in cultures exposed to OXC by either the plate incorporation or pre-incubation method. Therefore, OXC was not mutagenic in the bacterial reverse mutation assay.

7.1.5 Study title: (b) (4) **: Testing for Mutagenic Activity with Salmonella typhimurium TA 1535, TA 100, TA 1537 and TA 98 and Escherichia coli WP2uvrA**

Key study findings:

- (b) (4) **was not mutagenic in the bacterial reverse mutation assay.**

Study no.: 093-845
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 10/18/2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: (b) (4), PC040831, purity = 100%

Methods
Strains:
S. *typhimurium* TAl535 his G46 *rfa Δ*uvrB.
S. typhimurium TAl537 his C3076 *rfa Δ*uvrB.
S. typhimurium TA98 his D3052 *rfa Δ*uvrB- pKM101
S. *typhimurium* TA100 his G46 *rfa Δ*uvrB- pKM101
E. coli WP2uvrA trpE *rfa' Δ*uvrA

Concentrations used in definitive study: 5000, 1667, 500, 167, 50, 17 and 0 µg/plate

Basis of concentration selection: To determine toxicity, the following doses of (b) (4) were tested with and without metabolic activation: 5000, 1667, 500, 167, 50, 17 and 0 µg/plate. No concentration-dependent toxicity or precipitation was observed.

Negative controls: 10% DMSO

Positive controls:
Without S-9:
TAl535- Sodium azide (SA) l µg/plate
TAl537- 9-Aminoacridine (9AA) 80 µg/plate
TA98- Nitrofluorene (2NF) 1 µg/plate
TA100- Sodium azide (SA) 1 µg/plate
WP2 uvrA- N-Ethyl-N-Nitro-N-nitrosoguanidine (ENNG) 2 µg/plate

With S-9:
All strains exposed to 2-Aminoanthracene (2AA) 0.5-20 µg/plate. The activity of the S9 fraction was confirmed with 2-AAF, 4-AAF, benzo(a)pyrene and dimethylaminoazobenzene.

Incubation and sampling times: Both the plate incorporation and the pre-incubation methods were employed. In the plate incorporation method, plates were incubated at 37°C for 48-72 hours after the hardening of the agar. For the pre-incubation method, the pre-incubation period lasted for 20 minutes at 37°C. After addition of the pre-incubated mixture to Petri dishes, the dishes were incubated at 37°C for 48-72 hours.

Study validity: The results for the negative and positive controls for each strain were within the range of historic controls; therefore, the study is valid. The concentrations of (b) (4) employed are consistent with recommendations of regulatory guidances.

Results: No concentration-dependent increase in the number of revertants was observed in cultures exposed to (b) (4) by either the plate incorporation or pre-incubation method. Therefore, (b) (4) was not mutagenic in the bacterial reverse mutation assay.

7.2 *In vitro* Mouse Lymphoma Mutation Assay

7.2.2. Study title: **BIA 2-093: Mouse Lymphoma Cell Mutation Assay**

Key study findings:

- **Results of MLA tests suggest that BIA 2-093 is a very weak mutagen.**
- **Since the Sponsor did not qualitatively or quantitatively measure the metabolite profile of BIA 2-093 in the presence and absence of rat hepatic S9 fraction, it is unclear if proper concentrations of BIA 2-194, BIA 2-195 and OXC were represented in this assay.**

Study no.: 093-824
Study report location: EDR
Conducting laboratory and location: (b) (4)

Date of study initiation: 11/17/2000
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, 0000012976, 99.6%

Methods
Cell line: TK^+/ TK^- 3.7.2.C Mouse Lymphoma L5178Y Cells

Concentration used in definitive study:
Assay 1 (-S9): 100, 300, 500, 700, 900, 1300, 1500 µg/ml; Assay 2 (+S9): 300, 500, 700, 900, 1100, 1300, 1500 and 1700 µg/ml; Assay 3 (-S9): 200, 350, 500, 650, 800; Assay 4 (+S9): 600, 700, 800, 900, 1000 µg/ml

Basis of concentration selection: 100% cell death was observed at 2500 µg/ml in all assays. Therefore, the highest concentration was set below 2500 µg/ml.

Negative controls: 2 % DMSO

Positive controls: Without S9 (4 hours): 250 µg/ml Ethyl Methanesulfonate (EMS, large colony inducer); 15 µg/ml Methyl methanesulfonate (MMS, small colony inducer); With rat hepatic S9: 2.5 µg/ml 3-methylcholanthrene (3-MC)

Incubation and sampling times: The microwell variation of this assay was used by the Sponsor. Cells were incubated for 4 hours at 37^{o}C with the test article in the presence or absence of the S9 fraction. In addition, a separate experiment was performed with a 24 hour incubation time at 37^{o}C in the absence of the S9 fraction. Colony sizing was performed in all assays.

Study validity: The study is valid. All vehicle controls and positive controls expressed a mutation fraction that fell within the range of the historical controls. Further, the expected colony sizing ratios were obtained with EMS and MMS.

Results: Since the report for this set of studies was written in the year 2000, the authors were probably not aware of the International Workshop on Genotoxicity Testing (IWGT) Meeting Report published in 2006 [3]. The IWGT publication describes a method for determining the biological relevance of Mouse Lymphoma Assay (MLA) results. This method relies on the determination of two measures. The first measure, the Global Evaluation Factor (GEF), is a method for determining a threshold for characterizing borderline data as positive or negative. The GEF-adjusted mutation frequency is calculated by adding the GEF (126 for suspension MLAs) to the vehicle control mutation frequency (MF). If any value in the MLA exceeds the GEF-adjusted MF, the assay is considered positive. The GEF-adjusted MF was calculated by the Reviewer and can be found in Reviewer's Table 7.1 below. None of the GEF-adjusted MF values was exceeded in the MLAs. Therefore, the assays would not be considered positive according to the GEF method. However, the IWGT also recommends that, in conjunction with the GEF-adjusted MF, a trend analysis be performed on the data to determine if a dose response trend for an increase in mutations exists. The Sponsor did provide these data, which can be found in Reviewer's Table 7.1. According to the trend analysis, BIA 2-093 exhibits, in both assays, a

dose-related change in MF in the absence of S9. In the presence of S9, one of the two assays exhibits a significant trend. Therefore, BIA 2-093 was negative by the GEF method, but is generally positive by the trend analysis method. The interpretation of these conflicting results is that either the test compound is very weakly mutagenic or it contains or is metabolized to a mutagenic substance [3].

Unfortunately, the Sponsor did not determine the profile of metabolites in the MLA nor was each individual known metabolite (BIA 2-194, BIA 2-195 and OXC) tested in the MLA. Finally, a 24 hour incubation MLA was not performed because the 4 hour assays exhibited positive mutagenic activity.

Lymphoma Cell Mutation Assay	S9	GEF-adjusted MF ($x10^6$)	Max MF ($x10^6$)	Dose @ MAX MF (µg/ml)	Trend Analysis (p-value)
1	-	194	165	500 (*61%*)	0.016
3	-	199	192	800 (23%)	0.003
2	+	276	210	900 (26%)	0.14
4	+	209	177	800 (46%)	<0.001

Reviewer's Table 7.1: Results of MLA Testing the Genotoxicity of BIA 2-093. The GEF-adjusted MF is calculated by adding 126 to the MF obtained in the presence of the vehicle control as described by Moore et al [3]. GEF= Global Evaluation Factor, MF= Mutation Frequency. Max MF= the maximum MF observed at any concentration in the MLA. Values in parenthesis are the Relative Survival Percentage at the dose exhibiting the maximum MF. p<0.05 considered statistically significant.

7.3 *In vitro* chromosomal aberration assays in mammalian cells

7.3.1. Study title: BIA 2-093 Chromosomal Aberrations Assay with Human Peripheral Lymphocytes *In Vitro.*

Key study findings:

- **BIA 2-093 was not clastogenic in human peripheral blood lymphocytes (PBLs).**
- **Since the Sponsor did not measure the metabolite profile of BIA 2-093 in the presence and absence of S9, it is unclear if proper concentrations of BIA 2-194, BIA 2-195 and OXC were tested in this assay.**

Study no.: 093-827
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 7/29/1999
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, BN980220, Purity= 100.2%

Methods
Cell line: Human Peripheral Blood Lymphocytes

Concentration used in definitive study: 78, 156, 313, 625, 1250, 2500, 5000 µg/ml

Basis of concentration selection: Nine concentration levels were tested up to 5000 µg/ml to determine toxicity. In the absence of rat hepatic S9 fraction, cytotoxicity was observed after a 5 hour incubation with concentrations ≥ 1250 µg/ml. Cytotoxicity was observed after 5 hour incubation at 5000 µg/ml in the presence of S9. When incubated for 25 hours in the absence of S9, cytotoxicity was observed at 2500 and 5000 µg/ml. The concentrations used are consistent with current regulatory guidance.

Negative controls: 1% DMSO

Positive controls: Cyclophosphamide with S9 and Mitomycin C in absence of S9

Incubation and sampling times:

S9 Mix	Cultures Established	Test	Treatment Period	Recovery Period	Colcemid	Harvest
Presence of S9 mix	*ca* 48 h before exposure	Tests 1 and 2	0-5 h	5-26 h	26-29 h	29 h
Absence of S9 mix		Test 1	0-5 h	5-26 h	26-29 h	29 h
		Test 2	0-25 h	25-26 h	26-29 h	29 h
				25-50 h	50-53 h	53 h

Sponsor's Table: Incubation and Sampling Times

Study validity: Both positive controls increased the frequency of structural aberrations in lymphocytes. The negative controls were consistent with historical controls. Therefore, the study is valid.

Results: BIA 2-093 was not clastogenic in human peripheral blood lymphocytes in the presence or absence of S9 after a 5 or 24 hour incubation.

The Sponsor also demonstrated that BIA 2-093 did not cause polyploidy after prolonged treatment. The metabolite profile of BIA 2-093 was not examined in this study.

7.3.2. Study title: (b) (4) **Chromosomal Aberrations Assay with Human Peripheral Lymphocyte Cultures *In Vitro.***

Key study findings:
- (b) (4) **was not clastogenic in human PBLs.**
- (b) (4) **increased polyploidy in human PBLs at cytotoxic concentrations.**

Study no.: 093-846
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 10/16/2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: (b) (4), Batch PC040831, Purity = 99.8%

Methods
Cell line: Human Peripheral Blood Lymphocytes

Concentration used in definitive study: 156, 313, 625, 1250, 1700, 2100, 2500, 3750 and 5000 µg/ml.

Basis of concentration selection: Nine concentration levels up to 5000 µg/ml were used to determine toxicity. In the presence of rat hepatic S9 fraction, cytotoxicity was observed with a 5 hour incubation of ≥ 3750 µg/ml. Cytotoxicity was only observed at 5000 µg/ml in the absence of S9 after 5 hours of incubation. When incubated for 25 hours in the absence of S9, toxicity was observed at ≥ 2100 µg/ml. These concentrations are consistent with current regulatory guidance.

Negative controls: 1% DMSO

Positive controls: Cyclophosphamide with S9 and Mitomycin C in absence of S9

Incubation and sampling times:

S9 Mix	Cultures Established	Test	Treatment Period	Recovery Period (Includes 1 h wash)	Colcemid	Harvest
Presence of S9 mix	*ca* 48 h before exposure	Tests 1 and 2	0-5 h	5-26 h	26-29 h	29 h
Absence of S9 mix		Test 1	0-5 h	5-26 h	26-29 h	29 h
		Test 2	0-25 h	25-26 h	26-29 h	29 h
				25-50 h	50-53 h	53 h

Sponsor's Table: Incubation and Sampling Times

Study validity: Both positive controls increased the frequency of structural aberrations in lymphocytes. The negative controls were consistent with historical controls. Therefore, the study appears to be valid.

Results: (b) (4) was not clastogenic in human peripheral blood lymphocytes in the presence or absence of S9 after a 5 or 24 hour incubation. The metabolite profile of (b) (4) was not examined in this study. The Sponsor also examined the ability of (b) (4) to cause polyploidy in human PBLs. An increase in polyploidy frequency occurred at 1700 and 2100 µg/ml. However, cytotoxicity occurred at concentrations ≥ 1700 µg/ml.

7.4 *In vitro* Chinese Hamster Ovary Cell Cytogenetic Test:

7.4.1. Study title: ***In Vitro* Mammalian Cell Cytogenetic Test: Chinese Hamster Ovary Cells**

Key study findings:

- **BIA 2-093 was a clastogen in the CHO assay.**
- **The major chromosomal aberration observed in this study was chromatid deletion.**
- **As metabolites of BIA 2-093 were not measured, it is difficult to conclude from this study if only the parent compound is a clastogen or if its metabolites exhibit any clastogenic activity.**

Study no.: 093-825

Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 6/10/1998
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, lot BN 980220, Purity = 100.2%
Methods
Cell line: Chinese hamster ovary cells

Concentration used in definitive study:
In the presence of rat hepatic S9 fraction: 300, 750, 1500 and 3000 µg/ml
In the absence of S9: 300, 1500 and 3000 µg/ml

Basis of concentration selection: In the range finding experiment, 4.56, 22.8, 114.4, 572 and 2680 µg/ml of BIA 2-093 was tested. Precipitation was observed at 2680 µg/ml.

Negative controls: 1% DMSO

Positive controls: In absence of S9: Mitomycin C (0.15, 0.3, 0.5, 0.6 µg/ml); In presence of S9: Benzo(a)pyrene (5µg/ml) or cyclophosphamide (12, 15, 20 µg/ml).

Incubation and sampling times: In the first study, cells were treated for 3 hours in the presence and absence of S9. Harvesting occurred at 1.5 cell cycles. The second study was conducted using the same methodology except an additional set of cultures was sampled 24 hours after the first harvest. Two hours before all harvest time points, cells were treated with colcemid.

Study validity: The study is valid. The frequency of structural aberrations was increased in the presence of all positive controls. The negative controls were consistent with historical controls.

Results: In the range finding study, the highest concentration tested in the absence of S9 (2860 µg/ml) resulted in a 50% decrease in cell growth when compared to controls. In the presence of S9, growth at the HC was not affected.

The main assay demonstrated that BIA 2-093 is a clastogen in the absence and presence of the rat hepatic S9 fraction (Reviewer's Table 7.2). Acceptable, but variable, cell survival rates were observed in all experiments at the HD in the absence and presence of S9. The major form of chromosome aberration observed in BIA 2-093 exposed CHO cells is chromatid deletion (Reviewer's Table 7.3). Metabolites of BIA 2-093 were not measured in this study. Therefore, it is difficult to determine if the parent compound or its metabolites contribute to the observed clastogenicity.

Dose (µg/ml)	S9	Experiment 1 (% cells with aberrations)		Experiment 2-1 (% cells with aberrations)		Experiment 2-2 (% cells with aberrations)		Experiment 3 (% cells with aberrations)	
		A	B	A	B	A	B	A	B
0	-	6.5	6	5.5	4.5	2.5	2	2	2
300	-	2 (*73%*)	11 (*86%*)	5 (*127%*)	3 (*99%*)	----	----	3 (*91%*)	4 (*149%*)
750	-	----	----	----	----	----	----	4* (*162%*)	6* (*146%*)
1500	-	1 (*60%*)	10* (*88%*)	12* (*80%*)	36* (*103%*)	----	----	44* (*78%*)	26* (*146%*)
3000	-	3 (*60%*)	7 (*96%*)	12* (*87%*)	10* (*97%*)	16.9* (*54%*)	17* (*55%*)	----	----
PC	-	48* (*59%*)	52* (*125%*)	36* (*30%*)	40* (*46%*)	----	----	30.8* (*76%*)	60* (*73%*)
0	+	1.5	0	3.5	2	2	4.5	----	----
300	+	2 (*52%*)	1 (*92%*)	9* (*128%*)	1 (*129%*)	----	----	----	----
1500	+	0 (*33%*)	2 (*101%*)	2 (*128%*)	8* (*105%*)	----	----	----	----
3000	+	3 (*50%*)	0 (*75%*)	6* (*467%*)	8* (*353%*)	14* (*133%*)	40* (125%)	----	----
PC	+	32* (*40%*)	40* (*15%*)	52* (*18%*)	44* (*30%*)	----	----	----	----

*Reviewer's Table 7.2: BIA 2-093 is a Clastogen in the Chinese Hamster Ovary Cytogenetic Assay. The data presented are % of cells with aberrations (not including gaps). Values in parenthesis in each cell are the ratio of the mitotic index expressed as a percentage (treated/control), a measurement of cytotoxicity. *= significantly different from vehicle (information from Table 9 from Study 093-825). A and B are replicates from the respective experiments. Experiments 1, 2-1 and 3 involved 3 hour incubation with the test article with a harvest at 1.5 cell cycles later. Experiment 2-2 involved the same incubation time but the harvest time was 1.5 cell cycles plus 24 hours. PC= positive control (cyclophosphamide in presence of S9; Mitomycin C in the absence of S9).*

Dose	S9	Experiment 1 (chromatid deletion/100 cells)		Experiment 2-1 (chromatid deletion/100 cells)		Experiment 2-2 (chromatid deletion/100 cells)		Experiment 3 (chromatid deletion/100 cells)	
		A	B	A	B	A	B	A	B
0	-	2.5	0.5	1	1	1	0.5	0	0.5
300	-	0	0	4	1	----	----	0	3
750	-	----	----	----	----	----	----	2	3
1500	-	0	1	8	56	----	----	72	16
3000	-	1	2	12	2	1	10	----	----
PC	-	20	12	8	28	----	----	30.7	48
0	+	0	0	0	0.5	0	0.5	----	----
300	+	1	1	2	1	----	----	----	----
1500	+	0	0	0	0	----	----	----	----
3000	+	0	0	2	3	7	56	----	----
PC	+	28	24	12	24	----	----	----	----

Reviewer's Table 7.3: BIA 2-093 Causes Chromatid Deletion in Chinese Hamster Ovary Cells. The data presented are % of cells with aberrations (not including gaps). A and B are replicates from the respective experiments. Experiments 1, 2-1 and 3 involved 3 hour incubation with the test article with a harvest at 1.5 cell cycles later. Experiment 2-2 involved the same incubation time but the harvest time was 1.5 cell cycles plus 24 hours. PC= positive control (cyclophosphamide in presence of S9; Mitomycin C in the absence of S9).

7.4.2. Study title: ***In Vitro*** **Mammalian Cell Cytogenetic Test: Chinese Hamster Ovary Cells**

Key study findings:

- **OXC in the presence of S9 fraction resulted in robust chromosome exchange in CHO cells.**
- **BIA 2-093 was a clastogen in the CHO cell assay and it increases chromatid deletion in the absence, but not in the presence, of the rat hepatic S9 fraction.**
- **CBZ acted as a robust clastogen when incubated with CHO cells in the presence of the rat hepatic S9 fraction.**

Study no.: 093-826
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 10/19/1998
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: OXC, lot# 17/12/97, purity unknown; BIA 2-093, lot BN 980220, purity =100.2%; CBZ, lot #0019, purity unknown.

Methods
Cell line: Chinese hamster ovary cells

Concentration used in definitive study: OXC (-S9): 500, 2500, 500 µg/ml; OXC (+S9):390, 1950, 3900 µg/ml; BIA 2-093: 300, 150, 3000 µg/ml; CBZ: 150, 500, 1000 µg/ml

Basis of concentration selection: In a range finding study, Oxcarbazepine concentrations were 8, 40, 200, 1000 and 5000 µg/ml. CBZ concentrations were 1.6, 8, 40, 200 and 1000 µg/ml. 5000 µg OXC /ml, 1000 µg CBZ /ml and 3000 µg/ml BIA 2-093 precipitated upon incubation with CHO cells. Concentrations of BIA 2-093 were based on Study 093-825.

Negative controls: 1% DMSO

Positive controls: Mitomycin C (0.3 and 0.5 µg/ml) and cyclophosphamide (2 mg/ml)

Incubation and sampling times: In the first study, cells were treated for 3 hours in the presence and absence of rat hepatic S9 fraction. Harvesting occurred at 1.5 cell cycles. The second study was conducted using the same methodology except an additional set of cultures was sampled 24 hours after the first harvest. Two hours before all harvest time points, cells were treated with colcemid.

Study validity: The percentage of cells with structural aberrations was increased in the presence of all positive controls. The negative controls were consistent with historical controls. Therefore, the study is valid.

Results: As stated above, 5000 µg OXC /ml, 1000 µg CBZ /ml and 3000 µg/ml BIA 2-093 precipitated upon incubation with CHO cells and we considered to be the highest attainable concentration in the assay. OXC was shown to be a clastogen solely in the presence of the S9 fraction; the major aberration observed was chromosome exchange. CBZ was shown to increase the percentage of cells with chromosomal aberrations only in the presence of the S9 fraction.

Oxcarbazepine

Dose	Total	Aberrant	Mitotic	Gaps	Chromatid		Chromosome		Multiple	Numerical aberrations			% cells with aberrations		Relative
µg/ml	cells	cells	Index		deletion	exchange	deletion	exchange	aberrations	Poly	Endo	Hyper	with gaps	without gaps	Growth %
Without Metabolic Activation, 1st Harvest															
500	200	7	18.40	0	1	0	0	6	0	4	0	3	3.5	3.5	68
2500	200	8	15.60	0	2	1	0	5	0	2	0	3	4.0	4.0	78
5000	200	5	12.45	0	1	0	0	4	0	1	0	1	2.5	2.5	84
Control	400	21	7.85	10	2	0	2	7	0	10	0	1	5.3	2.8	100
MMC	50	34	2.25	5	19	21	6	1	2	5	0	1	68.0	64.0	
With Metabolic Activation, 1st Harvest															
390	200	19	7.25	3	3	0	1	14	0	5	0	4	9.5	8.5	139
1950	200	23	21.40	2	0	1	4	17	0	1	0	1	11.5	10.5	83
3900	200	11	26.30	2	1	0	1	8	0	3	0	0	5.5	5.0	62
Control	400	17	7.40	1	3	1	2	11	0	12	0	2	4.3	4.3	100
CPA	50	21	6.35	2	5	11	7	7	0	0	0	0	42.0	40.0	
Without Metabolic Activation, 2nd Harvest															
5000	200	5	8.35	0	0	1	0	5	0	14	0	10	2.5	2.5	
Control	400	7	7.20	2	0	2	0	3	0	2	0	1	1.8	1.3	
With Metabolic Activation, 2nd Harvest															
3900	200	40	7.40	8	7	1	0	36	0	107	0	86	20.0	18.0	
Control	400	24	7.43	6	1	2	7	12	1	3	0	5	6.0	4.8	

BIA 2-093

Dose µg/ml	Total cells	Aberrant cells	Mitotic Index	Gaps	Chromatid		Chromosome		Multiple aberrations	Numerical aberrations			% cells with aberrations		Relative Growth %
					deletion	exchange	deletion	exchange		Poly	Endo	Hyper	with gaps	without gaps	
Without Metabolic Activation, 1st harvest															
300	200	11	8.70	6	3	0	0	2	0	6	0	5	5.5	2.5	106
1500	200	50	11.95	29	25	5	0	4	0	7	0	3	25.0	13.5	54
3000	200	35	6.95	24	21	2	1	1	1	5	0	0	17.5	8.5	299
Control	400	21	7.85	10	2	0	2	7	0	10	0	1	5.3	2.8	100
MMC	50	34	2.25	5	19	21	6	1	2	5	0	1	68.0	64.0	
With Metabolic Activation, 1st Harvest															
300	200	11	7.25	2	1	0	1	9	0	3	0	0	5.5	5.0	112
1500	200	14	6.85	2	1	4	0	11	0	6	0	1	7.0	6.5	98
3000	200	22	5.05	2	2	2	1	17	0	2	0	1	11.0	10.0	85
Control	400	17	7.40	1	3	1	2	11	0	12	0	2	4.3	4.3	100
CPA	50	21	6.35	2	5	11	7	7	0	0	0	0	42.0	40.0	
Without Metabolic Activation, 2nd harvest															
3000	200	15	2.60	0	5	3	0	8	0	14	0	3	7.5	7.5	
Control	400	7	7.20	2	0	2	0	3	0	2	0	1	1.8	1.3	
With Metabolic Activation, 2nd Harvest															
3000	200	18	7.60	7	3	1	1	7	0	4	0	4.0	9.0	6.0	
Control	400	24	7.43	6	1	2	7	12	1	3	0	5.0	6.0	4.8	

Carbamazepine

Dose µg/ml	Total cells	Aberrant cells	Mitotic Index	Gaps	Chromatid		Chromosome		Multiple aberrations	Numerical aberrations			% cells with aberrations		Relative Growth %
					deletion	exchange	deletion	exchange		Poly	Endo	Hyper	with gaps	without gaps	
Without Metabolic Activation, 1st Harvest															
150	200	12	17.40	8	1	0	1	2	0	11	0	1	6.0	2.0	59
500	200	24	14.35	22	3	1	2	1	0	4	0	0	12.0	3.5	52
1000	200	18	12.15	12	1	0	3	3	0	10	0	2	9.0	3.5	72
Control	400	21	7.85	10	2	0	2	7	0	10	0	1	5.3	2.8	100
MMC	50	34	2.25	5	19	21	6	1	2	5	0	1	68.0	64.0	
With Metabolic Activation, 1st Harvest															
150	200	20	7.10	4	3	0	0	17	0	5	0	4	10.0	9.0	112
500	200	25	9.10	3	1	0	0	22	0	4	0	0	12.5	11.5	102
1000	200	17	7.75	1	2	1	1	13	0	5	0	2	8.5	8.0	78
Control	400	17	7.40	1	3	1	2	11	0	12	0	2	4.3	4.3	100
CPA	50	21	6.35	2	5	11	7	7	0	0	0	0	42.0	40.0	
Without Metabolic Activation, 2nd Harvest															
1000	200	20	3.20	3	1	6	0	12	0	18	2	2	10.0	8.5	
Control	400	7	7.20	2	0	2	0	3	0	2	0	1	1.8	1.3	
With Metabolic Activation, 2nd Harvest															
1000	200	10	6.90	5	0	1	0	5	0	2	0	4	5.0	2.5	
Control	400	24	7.43	6	1	2	7	12	1	3	0	5	6.0	4.8	

Sponsor's Tables: Chromosomal Aberration Analysis in CHO Cells

7.5 *In vivo* clastogenicity assay in rodent (micronucleus assay)

Study title: **Mouse Micronucleus Test**

Key study findings:

- **BIA 2-093 did not exhibit clastogenic activity in the *in vivo* mouse micronucleus assay.**
- **A temporary erythropoietic effect, with a NOEL of 320 mg/kg, was observed at 24 hours, but not 48 or 72 hours, after dosing.**

Study no.: 093-828
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 6/15/1998
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, BN 980220, purity = 100.2%

Methods:
Species: CD1 mice

Doses used in definitive study: Single dose of 320, 500 or 800 mg/kg by oral gavage.

Basis of dose selection: A dose range finding study was conducted using 500, 800, 1250 mg/kg. In this study, all mice (5 male and 5 female) exposed to 1250 mg/kg were euthanized *in extremis* within 4 hours of dosing due to severe test-article related symptoms (uncoordination, prostration, altered breathing and decreased body temperature). Only one of the five females and none of the males were euthanized *in extremis* in the 800 mg/kg dose group. Although animals in this dose group also exhibited severe test-article related symptoms (uncoordination, hypoactivity and decreased body temperature), all but the previously mentioned female survived to the end of the study (72 hours). Therefore, 800 mg/kg was considered to be an MTD and was chosen as the highest dose to be used in the definitive study.

Negative controls: 0.5% methylcellulose

Positive controls: Mitomycin C (0.4 mg/ml) in 0.9% saline

Incubation and sampling times: Mice were euthanized at 24, 48 or 72 hours after exposure to the test article. Bone marrow was sampled at each time point and prepared for analysis.

Study validity: Five animals were sampled per dose group per time point. A minimum of 2000 PCEs were counted for each animal. The positive control resulted in an increase in micronucleated PCEs (males =6.5 MNPCE/ 2000 PCE; females= 5.5 MNPCE/ 2000 PCE at 24 hours post dose) compared to the vehicle control. Measurement of plasma concentrations of BIA 2-093 and its metabolites (OXC and 10,11 dihydro metabolite) in a satellite group of mice demonstrated systemic exposure. It is important to note that the chiral analysis of metabolites was not performed in this study. The relative amounts of OXC and 10, 11 dihydro metabolite

achieved in this assay are consistent with previous characterization of mouse metabolism of BIA 2-093. All of these parameters and measurements are consistent with current regulatory guidance. Therefore, the study is valid.

Results: No increase in micronucleated PCEs was observed in any dose group at any time point in the study. This suggests that BIA 2-093 was not a clastogen in the mouse micronucleus assay. However, a temporary mitogenic or erythropoietic effect was observed in both the 500 mg/kg (62% increase in PCE/NCE ratio) and 800 (57% increase in PCE/NCE ratio) mg/kg dose groups at the 24 hour time point. This effect was not observed at 48 or 72 hours and may be due to the fact that, based on the half-life of 1.7-3 hrs in mice after a single acute dose (Study 093-806), substantial clearance of the test article and its metabolites would have occurred by 48 hours.

7.6 Other genetic toxicity studies

Study title: ***In vivo* Mouse Liver Unscheduled DNA Synthesis (UDS) Assay**

Key study findings:

- **BIA 2-093 did not increase the rate of UDS in the mouse.**

Study no.: 093-829
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 1/29/2001
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, 0000012976, purity = 99.6%

Methods:
Species: CD-1 Mice

Doses used in definitive study: 160 and 320 mg/kg BIA 2-093

Basis of dose selection: A dose ranging study using 320, 500, and 800 mg/kg was performed. Both mice dosed with 800 mg/kg were euthanized *in extremis* and severe clinical signs including decreased activity and prostration were observed in one of two mice receiving 500 mg/kg. Unlike the *in vivo* micronucleus assay where the highest dose used was 800 mg/kg, the highest dose used in the unscheduled DNA synthesis (UDS) assay was 320 mg/kg.

Negative controls: 0.5% hydroxypropylmethylcellulose in water.

Positive controls: N-nitrosodimethylamine (N-DMA)

Incubation and sampling times: Mice were euthanized two and sixteen hours after dosing and hepatocytes were isolated. Hepatocytes were incubated with ^{3}H-thymidine for four hours, washed and then allowed to incubate overnight before fixation and immersion in photographic emulsion.

Study validity: The Sponsor used the positive control recommended by OECD Guidance #486 for the short sampling time point (N-DMA at 2 hours). However, it is recommended by the OECD guidance that N-2-Fluorenylacetamide (2-AAF) [CAS no. 53-96-3] be used when sampling during later time points (12-16 hours). The Sponsor did sample at 16 hours post dose but relied on N-DMA as the positive control for this sampling time point. The negative and positive controls produced results consistent with the historical controls.

Finally, considering CD-1 mice were dosed as high as 800 mg/kg in the *in vivo* micronucleus assay (Study 093-828) without substantial mortality being observed, there is a clear inconsistency between Study 093-828 and the dose ranging study in the current study which demonstrates that mice could only tolerate doses as high as 320 mg/kg. No explanation for this disparity is provided by the Sponsor. It is the opinion of the Reviewer that the validity of this study is questionable, mostly due to the disparity in tolerated dose range in the CD-1 mouse between this study and Study 093-828.

Results: At the doses tested in this Study, hepatocytes from mice exposed to BIA 2-093 up to doses of 320 mg/kg did not exhibit increased thymidine labeling, *in vitro* (Table 1, Study 093-829). This finding suggests that BIA 2-093 did not cause UDS in the mouse. However, it is unclear if appropriate doses were used in this assay since previous experiments with CD-1 mice have demonstrated that this strain can tolerate up to 800 mg/kg BIA 2-093. In fact, OECD guidance #486 suggests that the highest dose be chosen "such that higher dose levels, based on the same dosing regimen, would be expected to result in lethality". No lethality was demonstrated at 500 mg/kg in the dose ranging study, yet the Sponsor did not include this as the high dose in the definitive study. The Reviewer suggests that the highest dose employed in the UDS assay should have been 500 mg/kg.

8. Carcinogenicity

Rat Carcinogenicity Waiver: In a previous review of IND 67466 written by Dr. Ed Fisher (dated 4/10/2007), it was recommended that the requirement for a rat carcinogenicity study of BIA 2-093 be waived since the study would be of little value to the human risk assessment of this compound. This decision was based on the observation that clinically relevant concentrations of the major active metabolite produced in humans, BIA 2-194, could not be achieved in rats given the propensity of this species to mainly metabolize the parent compound and its metabolites to OXC. A waiver of the rat carcinogenicity study was granted by the division on 7/20/2007.

Study title: **104 Week Oral (Gavage) Carcinogenicity Study in the Mouse**

Key study findings:

- **The carcinogenicity study design and analysis is adequate for assessment of the carcinogenic potential of BIA 2-093.**
- **Hepatocellular carcinoma and benign liver adenoma incidence was increased in male mice dosed with ≥ 250 mg/kg BIA 2-093 and in female mice dosed with 600 mg/kg.**
- **A dose-dependent increase in clinical signs such as convulsions, tremors, head tilting, circling, increased activity and agitation was observed in mice dosed with BIA 2-093.**
- **The incidence and severity of centrilobular hypertrophy and chronic hepatitis increased in a dose dependent manner in all male and female dose groups.**
- **A marked occurrence of extramedullary hematopoiesis in the spleen was observed in all male and female dose groups. A prominent dose-dependent increase in this finding was observed in animals surviving until the end of the study ("terminal kill").**
- **A majority of the deaths in this study were by euthanasia *in extremis* due to the severity of clinical signs.**
- **Increased incidence of degeneration of the germinal epithelium of the testes was observed in BIA 2-093 exposed male mice. A subset of these mice also exhibited epididymal aspermia.**
- **TK analysis of BIA 2-005 plasma levels demonstrated greater systemic exposure in males when compared to females receiving the same daily dose.**
- **The Sponsor has not provided convincing evidence that a mechanism of action similar to phenobarbital-induced hepatic neoplasia is the cause of the liver neoplasia observed in BIA 2-093 exposed mice.**

Adequacy of carcinogenicity study and appropriateness of test models: The study was adequately performed. Each study group consisted of a sufficient number of animals (greater than 10 mice) at the end of the study. The HD employed in this study was based upon the HD (650 mg/kg) used in the 13 week mouse, dose-ranging study. At 650 mg/kg, 2/24 males and 2/24 females were euthanized *in extremis* within the first week of the study. For the carcinogenicity study, the HD was set slightly lower (600 mg/kg) and was considered acceptable by the execCAC. Weight gain in LD, MD and HD males in this study was 93%, 90% and 74% of the vehicle controls,

respectively. Based on weight gain alone, the MD (250 mg/kg) would be considered the MTD in this study due to a reduction of 10% in body weight gain compared to controls. The test model (CD-1) used in this study was acceptable due to its ability to approximate the human metabolism of BIA 2-093.

Evaluation of tumor findings: The incidence of liver adenoma and hepatocellular carcinoma was clearly elevated in MD and HD male mice and HD female mice. Although these neoplasms are common in mice [4], the incidence of these tumor types was significantly increased in a dose-dependent manner in MD and HD male mice and in HD female mice, thereby suggesting that this increase is related to exposure to BIA 2-093. There was no other drug-related occurrence of neoplasia in this study.

ECAC meeting minutes for final study review: See Appendix

Study no.: 093-830
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 9/12/03
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093; Lot F030001; Purity 99.8%
CAC concurrence: Yes (8/28/03)
Methods:

Doses: 100 (LD), 250 (MD), 250 for week 1 and 600 for week 2-104 (HD) mg/kg/day; 54/sex/dose group in main study; 6 in control, 12 in all others in TK satellite groups
Basis of dose selection: A previous 13 week dose-ranging study (0, 150, 350, 500, 650 mg/kg BIA 2-093 in 0.5 % hydroxypropylmethylcellulose) was conducted in CD-1 mice. In this previous study, 2/24 males and 2/24 females dosed with 650 mg/kg were euthanized *in extremis* on Days 5 and 6 due to extreme clinical signs (hunched posture, piloerection, subdued behavior). BW gain in mice from this dose group was significantly increased in HD females and was similar to controls in males. According to ICH S1C, the dose of 650 would be greater than the MTD since it resulted in a significant reduction in the normal lifespan of the animals, in the form of humane euthanasia. The decision to start the HD mice in the 104 week study at an initial dose of 250 mg/kg before raising the dose to 600 mg/kg from weeks 2-104 was performed in an attempt to decrease the clinical signs associated with initiating dosing animals at a HD approximating the dose employed in the 13 week dose ranging study. The execCAC concurred with the selection of these doses for the carcinogenicity study on 8/28/2003.

Species/strain: Crl:CD-1 (ICR) BR VAF/PLUS
Route, formulation, volume: Oral Gavage, 0.5% hydroxypropylmethylcellulose, 10 ml/kg
Frequency of dosing: Once daily
Age: 28-35 days old on arrival, dosing began after a 17 day acclimation period
Animal housing: Males were housed 3/cage; Females 3-5/cage in grid bottomed cages. Animals were provided environmental enrichment.

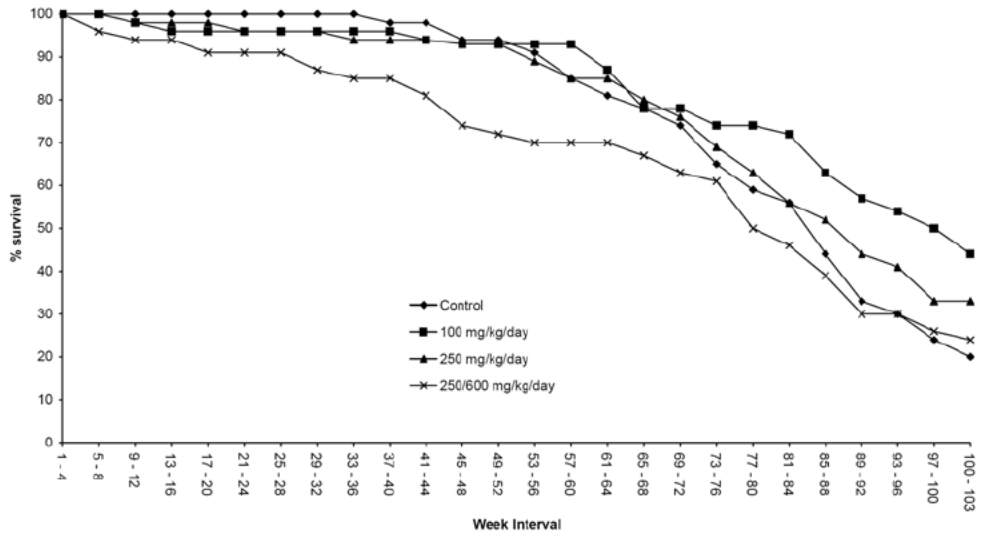
Restriction paradigm for dietary restriction studies: Water and feed were supplied *ad libitum*.
Dosing solution analyses/Drug stability and homogeneity: No test article was detected in vehicle control dosing formulations. All test article formulations were within ± 14% of the nominal concentration.
Dual controls employed: Dual controls not employed
Interim sacrifices: No interim sacrifices were performed.
Deviations from original study protocol: None of the deviations detailed in Section 3.1 would be expected to affect the validity of the study.

Observation Times and Results

Mortality: All surviving males were euthanized during week 104, after consultation with the FDA. Females were continued for the full 104 scheduled weeks of the study and were euthanized in week 105. All dose groups had >10 animals remaining in the study at the time of termination.

Survival differed between male and female mice (Sponsor's Figure 1 and 2; Study 093-830). Specifically, the survival rate was decreased in HDM. According to the Statistical Review and Evaluation of Study 093-830 [5], this difference between HDM and vehicle controls was significant earlier in the study. However, by week 103, no significant difference in the survival curves was observed between HDM and vehicle control males. Survival in LDM was significantly increased at week 103 when compared to vehicle controls. Although HDF exhibited higher mortality in comparison with vehicle controls between week 1 and week 42, no statistically significant difference in mortality rate was observed for any female dose group when compared to controls.

Figure 1 - Survival (% of original group size) - males



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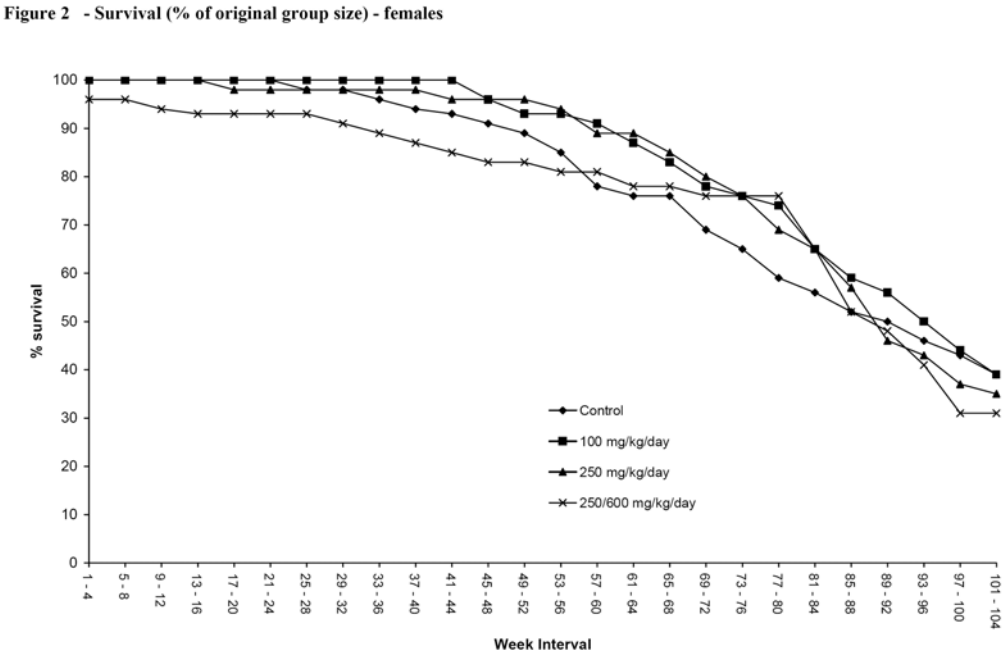
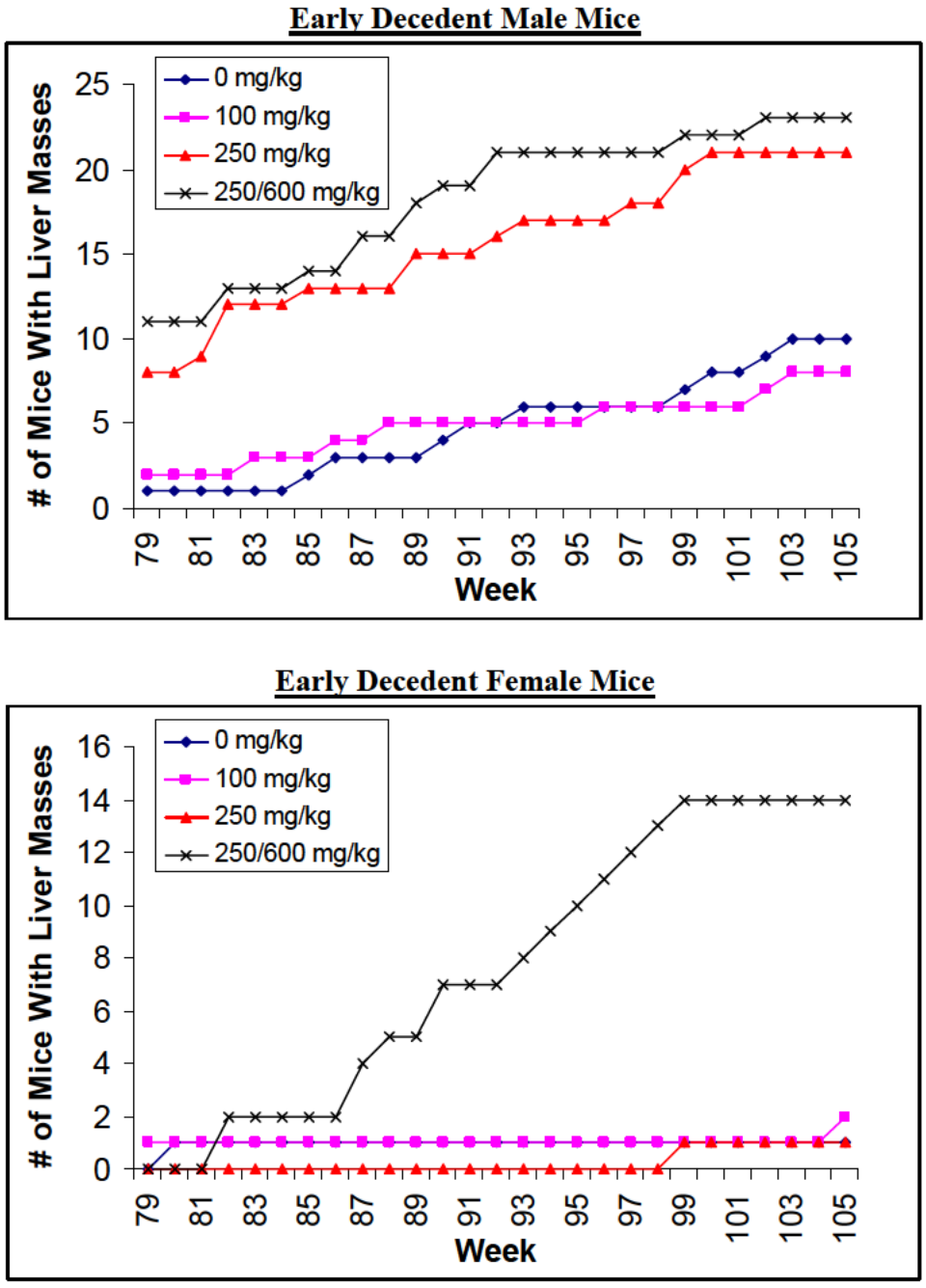


Sponsor's Figures 1 & 2: Animal Survival

In general, a majority of the deaths (60-70 % males, 70-90 % females) observed in this study were due to euthanasia *in extremis* in response to severe clinical signs such as cold body surface, breathing abnormalities, abnormal gait, decreased activity, piloerection, distended abdomen and various skin lesions. Since all of these clinical signs besides the skin lesions occurred in a dose-dependent manner, they are considered to be test-article related. In addition, test-article related clinical signs similar to those described by the Sponsor in studies with beagle dogs as "seizure-like" were observed in early decedents dosed with BIA 2-093. Specifically, tremors occurred in 1, 2, 1 and 4 of the control, LD, MD and HD animals, respectively. If tremors are grouped together with convulsions, head tilting, circling, increased activity and agitation, a clear dose-dependent increase in incidence of seizure-like symptoms was observed (1, 2, 4, 9 animals in the control, LD, MD and HD groups, respectively). However, it is important to note that the Sponsor did not report seizures in mice dosed with BIA 2-093.

An increase in the incidence of early decedents with liver masses was observed in MDM and HDM and HDF (Reviewer's Figure 8.1). As observed in the 13 week study, HDMs at week 13 exhibited greater levels of BIA 2-005 than HD females in this study. At week 26, both MDM and HDM had higher levels of BIA 2-005 than MDF and HDF.



Reviewer's Figure 8.1: Cumulative Incidence of Liver Masses in Early Decedents.

Clinical signs: An increased incidence of cold body surface and slow breathing in HD males and females, as well as decreased activity in HDM and MDF and HDF, is consistent with observations made in the respiratory and FOB safety pharmacology studies performed by the Sponsor. Furthermore, gait disturbances also occurred in MD and HD males and females.

Clinical signs such as circling (M= 14, F=36) and head tilting (M=19, F=27) were observed in HD animals. Additional clinical signs, such as convulsions and tremors, were not observed in controls but did occur in a dose dependent manner in all female, but not male, dose groups (Convulsions= 0, 1, 1, 3 and Tremors= 0, 2, 4, 38 in controls, LD, MD and HD, respectively).

While it is important to acknowledge that the Sponsor does not distinctly classify these observations as seizures, similar clinical signs have been described by the Sponsor as "seizure-like" in the non-rodent studies.

Distended abdomen, potentially a consequence of increased incidence of liver masses, was observed to increase in a dose dependent fashion in MDM and HDM. HDF exhibited an increased incidence of in abnormal abdomen color, but an increased incidence of distended abdomen is not described by the Sponsor in females.

Overall, it is important to appreciate that the Sponsor expanded the monitoring of HD mice due to the increased incidence of clinical symptoms in this group. Initially, up to week 16, animals from all dose groups were observed once a week at predose, 0.5 hours and 3 hours post dose. After 16 weeks, this observation occurred once every four weeks. However, the Sponsor states that the monitoring for the HD group alone was expanded but does not provide specific details on the expanded observational scheme. Since the observational scheme differed for the HD group, this may have resulted in underreporting of clinical signs in control, LD and MD mice.

Body weights: Although all dose groups gained weight during the study, group mean BW gain was dose-dependently reduced in males at all doses, with the reduced BW gain from week 1-104 in HDMs reaching statistical significance (BW gain Week 1-104= 16.3, 15.3, 14.7 and 12 g in Control, LD, MD and HD male mice, respectively). The Sponsor's BW curves for males and females are provided below. From these curves, it is obvious that the significantly reduced BW gain in HDM is due to the lower BW observed for this group from Week 44-104. This effect was not as pronounced in HDF.

Figure 4 - Bodyweights (g) - group mean values - Weeks 1 to 104 - males

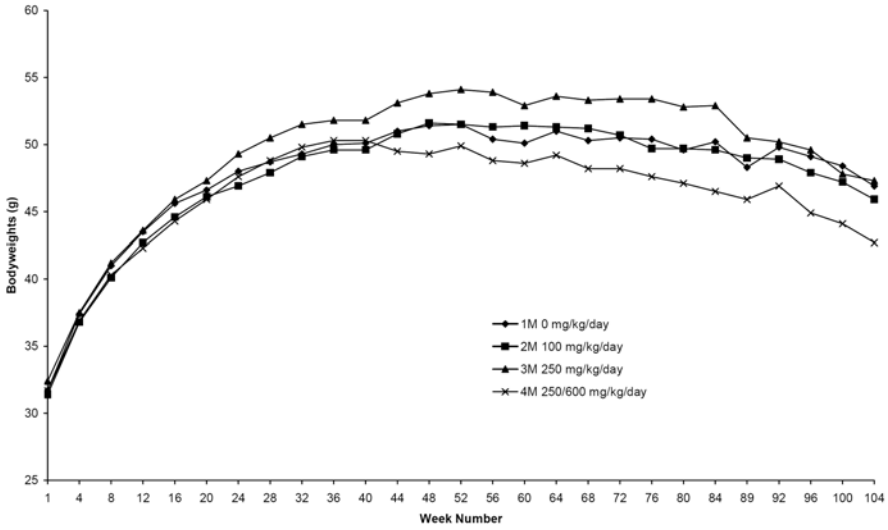


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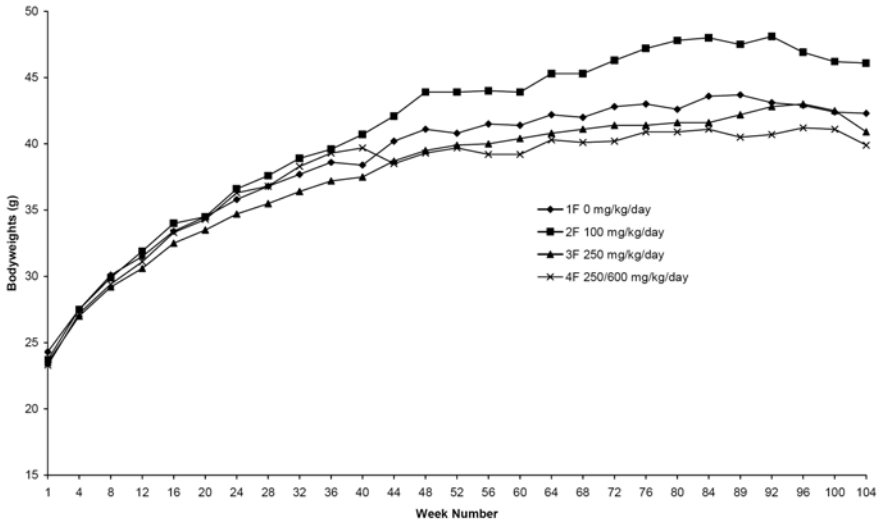
Figure 6 - Bodyweights (g) - group mean values - Weeks 1 to 104 - females



Sponsor's Figures 4 & 6: Male and Female BW curves

Food consumption: Over the entire treatment period, mean food intake was similar in all treatment groups to control. Food consumption was significantly increased in HDMs (10-15%) from week 53 to the end of the study and was increased in HDFs (20%) from weeks 80 until the end of the study.

Ophthalmoscopy: No test article related abnormalities.

Hematology: No consistent alterations related to the test article were observed. Reticulocyte count, APTT and platelet count, all measures affected by BIA 2-093 exposure in other non-clinical models, were not assessed in this study.

Gross Pathology: As observed in the early decedents, the number of animals with liver masses was increased in the male MDM and HDM mice and in the HDF mice when compared to all other groups.

Factors Contributing to Death: It was determined by the Study Pathologist that liver neoplasia was the predominant cause of death in male mice. This effect increased in a dose dependent manner in the MDM and HDM groups. In females, the major factor contributing to death was hematopoietic neoplasia in the form of follicular center cell lymphoma and histiocytic sarcoma. This finding did not exhibit a dose-related incidence. Liver tumors in males and lymphoid tumors in females are ranked as tumors that occur with the greatest spontaneity in mice [4]. The presence of a dose dependent increase in liver tumors in MDM and HDM and HDF suggests that these findings are related to the test article.

Histopathology: Peer Review: Yes; Adequate Battery: Yes

Neoplastic: According to FDA's Statistical Review, the only tumor findings that achieved statistical significance were increases in liver adenomas and hepatocellular carcinomas in MD males, HD males and HD females [5].

A) Liver: Incidence and multiplicity of benign liver adenoma (12/54, 18/54, 26/54, 23/54; control, LD, MD, HD, respectively) and hepatocellular carcinoma (5/54, 10/54, 29/54, 32/54; control, LD, MD, HD, respectively) were increased in MDM and HDM mice in a dose dependent manner. Occurrence of these findings did not differ between LDM and control mice. In female mice, the incidence of liver adenoma (0, 2, 1, 15; control, LD, MD, HD, respectively) and hepatocellular carcinoma (0, 0, 2, 22; control, LD, MD, HD, respectively) was markedly increased in the HDF group only.

Text Table 9: Liver tumour incidence table

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
Dose level (mg/kg/day)	0	100	250	250/600	0	100	250	250/600
Malignant Carcinomas								
One	3	9	18	13	0	0	2	9
Two	2	1	8	11	0	0	0	5
Three	0	0	2	7	0	0	0	1
Four	0	0	1	1	0	0	0	0
Total#	***7***	***11***	***44******	***60******	***0***	***0***	***2***	***22******
Benign Adenomas								
One	8	14	13	14	0	2	1	10
Two	3	4	7	5	0	0	0	1
Three	1	0	1	3	0	0	0	1
Four	0	0	3	0	0	0	0	0
Five	0	0	1	1	0	0	0	0
Six	0	0	1	0	0	0	0	0
Total#	***17***	***22***	***53****	***38****	***0***	***2***	***1***	***15******
No of Animals with Carcinomas#	5	10	29***	32***	0	0	2	15***
No of Animals with Adenomas#	12	18	26**	23**	0	2	1	12**
No of Animals with Hepatic Neoplasia	15	24	38	40	0	2	2	23

- statistically analysed *=p<0.05 **=p<0.01 ***=p<0.001

Sponsor's Summary Table 9: Hepatic Neoplasia Incidence

Non neoplastic:

A) Liver: As summarized in the Sponsor's table below (Text Table 10), the incidence and severity of centrilobular hypertrophy and chronic hepatitis increased in a dose dependent manner in all male and female dose groups. A significant increase in the incidence of regenerative hyperplasia and focal necrosis was observed in MDM and HDM. HDF mice exhibited a slight increase in the incidence of regenerative hyperplasia, but not necrosis. Vacuolation of the periportal hepatocytes was observed in all female, but not male, mouse dose groups.

Text Table 10: Non-Neoplastic Liver Findings Related to Treatment

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
Dose level (mg/kg/day)	0	100	250	250/600	0	100	250	250/600
Centrilobular Hypertrophy	0	14***	22***	21***	6	12	13(*)	21***
Chronic Hepatitis	6	16	21**	28***	6	8	13	17*
Regenerative Hyperplasia	0	1	5(*)	4(*)	0	0	0	3
Focal Necrosis	0	2	11**	9**	5	4	5	4
Periportal Hepatocyte Vacuolation	0	0	0	0	0	7*	5*	5(*)

(*) = p<0.1 *=p<0.05 **=p<0.01 ***=p<0.001

Sponsor's Summary Table 10: Hepatic Histopathology

B) Spleen: Although there was a marked occurrence of extramedullary hematopoiesis observed in both male and female vehicle control mice, the incidence was elevated in all BIA 2-093 dose groups (Male: 20/54, 32/54, 31/54, 36/54; Female: 22/52, 27/54, 31/54, 37/54; Control, LD, MD HD, respectively). When analyzed by time of euthanasia ("terminal kill" vs. "early decedent"), the increased incidence becomes more pronounced in animals that survived for the entire length of the study ("terminal kill" Males: 2/11, 18/24, 16/18, 13/13; Females= 6/21, 11/19, 15/19, 12/17 vs. "early decedent" Males: 18/43, 14/30, 15/36, 23/41; Females= 16/33, 16/35, 16/35, 25/37; control, LD, MD, HD, respectively).

C) Reproductive Organs: The Sponsor attributes the occurrence of these findings to "altered hormonal status" but does not provide data regarding male or female hormone levels in mice dosed with BIA 2-093.

Males: An increased incidence of minimal to moderate degeneration of the germinal epithelium of the testes was observed at all doses (11/54, 20/54, 20/54, 16/54; control, LD, MD, HD, respectively). A subset of these mice also exhibited epididymal aspermia (4/54, 11/54, 7/54, 6/54; control, LD, MD, HD, respectively). This finding was also observed in male rats exposed for 26 weeks to BIA 2-093 (Study #093-810).

Females: A slight increase in ovary luteal cell hyperplasia (1/53, 2/53, 4/53, 5/54; control, LD, MD, HD, respectively) and a decreased incidence of secretory activity in the mammary gland was observed (Mammary secretory activity: 17/54, 10/54, 10/54, 1/54, Control, LD, MD, HD, respectively).

D) Gall bladder: Incidence of gall bladder distension was increased in all male dose groups (7/53, 16/54, 14/51, 13/54, control, LD, MD, HD, respectively) in the absence of any microscopic correlates.

E) Adrenals: Male, but not female, mice exhibited an increased incidence of medullary hyperplasia (13/54, 14/54, 17/54, 24/54, control, LD, MD, HD, respectively). The Sponsor suggests that this finding, like the reproductive organ findings, is due to an

alteration in hormonal status. However, the Sponsor does not provide evidence for this statement.

Toxicokinetics: BIA 2-093 or its metabolites were not detected in control animals. TK parameters were not calculated in this study. Plasma concentrations were measured at one and four hours post dose and are stated as ng/ml BIA 2-093, BIA 2-005 or OXC. Plasma concentrations of the major metabolite, BIA 2-005, are less than dose proportional between 100 and 250 mg/kg but are roughly dose proportional between 250 and 600 mg/kg in males at week 13 and week 26. In females, plasma concentrations are roughly dose proportional at week 13 and week 26. Plasma concentrations in males are higher than females at week 13 and week 26 and are roughly similar to the C_{max} observed in the 13 week dose ranging study in mice.

Text Table 7: Group mean plasma level of the test article and metabolites in Weeks 13 and 26

Group/sex		6M	7M	8M	6F	7F	8F
Dose level (mg/kg/day)	Timepoint hours post-dose	100	250	600	100	250	600
BIA 2 - 093 (ng/mL)							
Week 13	1	BLQ*c	BLQ*c	BLQd	BLQ*c	BLQ*d	1035*^
	4	BLQ*d	BLQ*d	BLQ*d	BLQ*d	BLQ*d	BLQ*d,e
Week 26	1	BLQ	BLQ	62.2*	BLQ	159.5**	238*
	4	BLQ	BLQ	BLQ	BLQ	BLQ	52.5*
Oxcarbazepine (ng/mL)							
Week 13	1	695**	BLQ**	BLQ**	BLQ**	BLQ**	BLQ**
	4	BLQ**	BLQ**	BLQ	BLQ**	BLQ**	130**
Week 26	1	1025	1151	2751	541	2177	3020
	4	746	2152	4533	1178	3163	5118
BIA 2 - 005 (ng/mL)							
Week 13	1	32301	40146	85855	27205	50427	74331
	4	15849	18412	24776	7302	5334	50853
Week 26	1	25107	32885	76424	15766	27366	46112
	4	9361	20754	38753	3929	11050	26176

Sponsor's Summary Table 7: Toxicokinetics

9. Reproductive and Developmental Toxicology

Reviewer's Summary:

- **A NOEL for decreased number of implantation sites and live embryos per female could not be determined.**
- **BIA 2-093 decreased the number of estrous cycles observed in female rats exposed to >125 mg/kg BIA 2-093.**
- **Mouse fetuses exposed to BIA 2-093 *in utero* exhibited an increased incidence of cleft palate and exencephaly at doses that were not maternally toxic. No NOEL was defined for these malformations. Skeletal abnormalities, such as incomplete ossification, were observed at doses that were maternally toxic.**
- **Rabbit fetuses exposed to BIA 2-093 *in utero* exhibited an increased incidence of extra presacral vertebrae and irregular ridging of the palate. No NOEL was defined for these malformations. These abnormalities were observed at doses that were not maternally toxic.**
- **Eye and ear opening were delayed in mouse pups born to dams exposed to BIA 2-093.**
- **Preputial separation and vaginal perforation were delayed in rat and mouse pups born to dams exposed to BIA 2-093. However, fertility of the pups was not affected.**

9.1 Fertility and early embryonic development

9.1.1 Mouse

9.1.1. A. Study title: **Toxicokinetic Study in the Pregnant Mouse**

Key study findings:

- **Decreased activity and unsteady gait were consistently observed in pregnant dams exposed to BIA 2-093 at ≥ 350 mg/kg.**
- **In pregnant dams exposed to ≥ 350 mg/kg, BW gain was reduced.**
- **BIA 2-093 plasma levels were below the limit of quantification presumably because of its extensive metabolism to BIA 2-194, BIA 2-195 and OXC.**
- **BIA 2-194 is the main circulating metabolite of BIA 2-093 in pregnant mice.**
- **OXC and BIA 2-194 plasma levels increased in a dose proportional fashion. However, BIA 2-195 increased in a greater than dose-proportional manner from 350 to 650 mg/kg/ day BIA 2-093.**

Study no.: 093-847
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 3/5/2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, 25570-1-3, purity = 99.3%
Methods:

Doses: 150, 350, 650 mg/kg/day
Species/strain: Timed mated CD-1 mice
Number/sex/group: 24 females/ group

Route, formulation, volume, and infusion rate: Oral gavage; once daily on GD 6-15; 5 ml/kg
Dosing solution analyses/Drug stability and homogeneity: Test article was formulated in 0.5% hydroxypropylmethylcellulose; drug solutions were stable throughout the study and were within 100.2-106.2% of the theoretical concentration.
Study design: Blood samples were collected on day 6 and day 15 (0.5, 2, 4, 8, 12 and 24 hours post dose on each day)
Parameters and endpoints evaluated: plasma BIA 2-093, BIA 2-194, BIA 2-195, OXC

Results:

Mortality: No deaths observed.

Clinical signs: Females dosed with 150 mg/kg had no clinical signs. In the group receiving 350 mg/kg, 12 out of 24 mice exhibited altered gait and decreased activity that lasted for up to 6.5 hours post dose. All animals receiving 650 mg/kg has decreased activity, unsteady gait, slow breathing and flat posture lasting from one hour after dosing until the time of the next dose.

Body weight: Beginning on Day 6 and lasting until the end of the study, body weight was decreased in pregnant dams exposed to 350 and 650 mg/kg/day BIA 2-093. Overall BW gain was 18% lower in HDF than in vehicle exposed animals.

Pregnancy Data: No dose-related effect on the % of dams pregnant in each dose group was observed at the end of the study.

Toxicokinetics: BIA 2-093 plasma levels were below the limit of quantification presumably because of its extensive metabolism to BIA 2-194 and OXC. The half life for the BIA 2-093 metabolites ranged from 1.4 to 4 hours. OXC and BIA 2-194 plasma levels increased in a dose proportional fashion. However, BIA 2-195 increased in a greater than dose-proportional manner from 350 to 650 mg/kg/ day BIA 2-093. By far, the greatest systemic exposure was to BIA 2-194 followed by OXC and BIA 2-195.

Day 6

Analyte	Dose (mg/kg/day)	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-t} (hr*ng/mL)	AUC_{0-8} (hr*ng/mL)	$t_{1/2}$ (hr)
BIA 2-093	150	0	NC~	0	NC~	NC~
BIA 2-093	350	0	NC~	0	NC~	NC~
BIA 2-194	150	47500	0.500	130000	124000	NC+
BIA 2-194	350	95400	0.500	375000	333000	2.34
BIA 2-195	150	0	NC~	0	NC~	NC~
BIA 2-195	350	155	4.00	669	669	NC+
Oxcarbazepine	150	8490	2.00	47200	43000	NC+
Oxcarbazepine	350	18400	4.00	113000	99800	2.15

Day 15

Analyte	Dose (mg/kg/day)	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-t} (hr*ng/mL)	AUC_{0-8} (hr*ng/mL)	$t_{1/2}$ (hr)	R_O
BIA 2-093	150	0	NC~	0	NC~	NC~	NC
BIA 2-093	350	0	NC~	0	NC~	NC~	NC
BIA 2-093	650	0	NC~	0	NC~	NC~	NC
BIA 2-194	150	25700	0.500	49900	49900	1.38	0.402
BIA 2-194	350	33300	0.500	117000	94900	3.89	0.285
BIA 2-194	650	51300	0.500	268000	216000	4.12	NC
BIA 2-195	150	13.0	2.00	NC&	NC&	NC+	NC
BIA 2-195	350	41.2	2.00	112	NC^	NC+	0.345$
BIA 2-195	650	133	2.00	788	788	NC+	NC
Oxcarbazepine	150	6720	0.500	21600	21600	1.40	0.501
Oxcarbazepine	350	7440	0.500	38300	30800	3.87	0.309
Oxcarbazepine	650	13000	0.500	91200	71900	3.66	NC

NC+ Not calculated; a terminal monoexponential decline could not be unambiguously identified.
NC~ Not calculated; all plasma concentrations below the lower limit of quantification.
NC& Not calculated; only one measurable plasma concentration therefore estimation of AUC (AUC_{0-t} = 9.77 hr*ng/mL) not considered reliable.
NC^ Not calculated; plasma concentrations only measurable up to 4 hr post-dose for BIA 2-195 at 350 mg/kg/day.
NC Not calculated.
$ AUC_{0-4} used for calculation of R_O (AUC_{0-4} on Day 6 = 325 hr*ng/mL; AUC_{0-4} on Day 15 = 112 hr*ng/mL).

Sponsor's Table: Toxicokinetics

9.1.1. B. Study title: **Oral (Gavage) Fertility and Early Embryonic Developmental Study in the Mouse**

Key study findings:

- **A NOEL for decreased number of implantation sites and live embryos per female could not be determined since the effect was present at all doses.**
- **Decreased activity, unsteady gait and other clinical symptoms were observed in mice receiving 350 and 650 mg/kg BIA 2-093.**
- **The number of females not pregnant was increased in the 350 and 650 mg/kg dose groups when compared to the control animals.**
- **Assessment of sperm viability and sperm counts in the epididymis, as recommended in ICH S5A (4.1.1.h), was not performed. Furthermore, histopathological analysis of the testes to assess the effect of BIA 2-093 on spermatogenesis was not performed in this study.**
- **Overall, the results of this study suggest that BIA 2-093 impairs fertility in the mouse.**

Study no.: 093-831
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 2/8/2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, 25570-1-3, Purity = 99.3%
Methods

Doses: 0, 150 (LD), 350 (MD), 650 (HD) mg/kg
Species/strain: Crl: CD-1 (ICR) BR VAF/PLUS mice
Number/sex/group: 30 mice/ sex /group
Route, formulation, volume, and infusion rate: Oral gavage, 0.5% hydroxypropylmethylcellulose, 5 ml/kg
Dosing solution analyses/Drug stability and homogeneity: No test article was detected in the control formulation. Drug solutions were determined to be homogenous with less than a 10% difference between the top, middle and bottom of a stock solution. All samples were within 85-115% of the nominal concentration.
Satellite groups used for toxicokinetics: None
Study design: Males were dosed for 28 days prior to mating and females 14 days prior to mating until day 6 of gestation.

Results:
Mortality: One HDF was found dead on Day 2. This death was considered by the Sponsor to be treatment-related. This animal exhibited flat posture, decreased activity, unsteady gait, palpebral closure and slow breathing.

Clinical signs: All MDFs, HDFs and HDMs exhibited decreased activity, unsteady gait, slow breathing, flat posture and palpebral closure. These symptoms lasted for up to 24 hours after dosing on Day 1 but reduced in duration to 1 to 5 hours as the study progressed. The duration of

the effects at the MD was shorter (0.75-3.5 hours) than at the high dose. Clinical signs were not observed in vehicle or LD mice.

Body weight: No statistically significant alterations in weight gain were observed in LDM and MDMs. Before mating, all females exhibited a decrease in body weight gain until day four of dosing. MDFs and HDFs had lower mean BW gain (9% and 18%, respectively) when compared to controls between GD 7-13.

Food consumption: No treatment-related effects were observed on food consumption.

Necropsy: No dose-related abnormalities in the macroscopic gross pathology analysis were observed in any dose group. Histopathological analysis was not performed on mouse testes. Determination of sperm viability, sperm counts in the epididymides and corpora lutea counts, as recommended in ICH S5A [6], were not performed in this study.

Fertility parameters: No treatment-related effects were observed in any of the dose groups on the copulation index, fertility index, or number of sperm plugs after pairing. However, a dose-dependent decrease in the number of implantation sites and live embryos per female was observed in all dose groups (Sponsor's Table 16; Study 093-831). Therefore, the NOEL for these effects is <150 mg/kg.

Test article :	Control	BIA 2-093		
Dose (mg/kg/day) :	0	150	350	650
	Group 1	Group 2	Group 3	Group 4
Number of females with implantations at scheduled kill	29	29	25	27
Number of implantations	405	377	314	331
Mean number per female#	14.0	13.0*	12.6**	12.3***
Standard deviation	2.0	1.8	1.4	1.3
Number of early embryo/foetal deaths	18	19	16	15
Number of dead embryos	2	0	0	0
Mean % post-implantation loss#	4.8	5.1	5.2	4.5
Number of live embryos	385	358	298	316
Mean number per female#	13.3	12.3*	11.9**	11.7**
Standard deviation	2.1	2.0	1.8	1.4
Mean % of implantations	95.2	94.9	94.8	95.5

= statistically analysed *=p<0.05 **=p<0.01 ***=p<0.001

Sponsor's Table 16: Fertility Parameters in Mice Dosed with BIA 2-093

9.1.2 Rat

9.1.2. A. Study title: **Oral (Gavage) General Reproductive Performance Dose Ranging Study in the Rat**

Key study findings:

- **Maternal toxicity in the form of decreased body weight during pregnancy was observed at 250 mg/kg; the NOAEL was 150 mg/kg.**
- **Fertility and mating performance were not affected in BIA 2-093 exposed rats.**
- **Plasma levels of BIA 2-093 and its metabolites were not assayed in this study.**

Study no.: 093-832
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 11/17/2000
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, 0000012976, Purity = 99.7%
Methods:

Doses: 0, 20 (LD), 75 (MD1), 150 (MD2), 250 (HD) mg/kg/day BIA 2-093
Species/strain: HSD: Sprague-Dawley rat
Number/sex/group: 6
Route, formulation, volume, and infusion rate: Oral gavage, 0.5% hydroxypropylmethylcellulose, 5 ml/kg
Dosing solution analyses/Drug stability and homogeneity: No test article was detected in the control formulation. The 4 mg/ml and 15 mg/ml solutions were homogenous, with less than 10% difference between the top, middle and lower portion of the suspension. The 30 and 50 mg/ml solutions fell outside the accepted tolerance of 10% (63.7% and 85.7% of the nominal concentration, respectively). This would be expected to result in differences between the nominal and actual doses administered to the HD group.
Study design: Males were dosed for two weeks before mating and were continued on the dosing regimen until the time of necropsy (57 days in total). Females were dosed for two weeks before mating and were continued on the dosing regimen until postpartum day 7.

Results:

Mortality: No test article related deaths were observed in any of the dose groups.

Clinical signs: No test article related clinical signs were observed in any of the dose groups.

Body weight: Males: MD1Ms exhibited decreased BW gain (12%) throughout the course of the study when compared to controls. No other dose group exhibited an alteration in BW or BW gain.

Females: During the premating period, there was no test article related effect on BW gain. However, HDFs exhibited a 31% decrease in BW gain from GD 14-20. During the post-partum phase, MD2Fs and HDFs exhibited decreased BW gain (47% and 16%, respectively).

Food consumption: Food consumption was not affected in males exposed to BIA 2-093. HDFs exhibited a slight, but significant (14%) decrease in food consumption between GD 7-14. Decreased food consumption (~20%) was also observed in rats exposed to ≥ 75 mg/kg during lactation.

Necropsy: No obvious test article related gross pathology abnormalities were observed in males, females or pups born to treated females.

Fertility parameters: The mean number of days taken to mate, the number of sperm plugs after pairing, copulation index, fertility index, gestation index, mean duration of gestation, pup sex ratio, mean live birth index, mean viability index and cumulative survival index were not altered in rats exposed to BIA 2-093.

9.1.2. B. Study title: **Oral (Gavage) Fertility and Early Embryonic Development Study in the Rat**

Key study findings:

- **The design and execution of this pivotal study were adequate.**
- **Due to a decrease in the number of estrous cycles observed in female rats exposed to 250 mg/kg, the NOEL for reproductive toxicity in rats was determined to be 125 mg/kg in this study.**
- **Decreased, but not statistically significant, number of corpora lutea, implantations and live fetuses were observed in the 250 mg/kg dose group.**
- **Maternal water intake was increased in all dose groups.**
- **Systemic toxicity consisting of distended GI tract containing an unidentified firm green material was observed in females exposed to 125 and 250 mg/kg BIA 2-093.**

Study no.: 093-833
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 2/9/2001
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, 0000012976, Purity = 99.7%
Methods -

Doses: 65 (LD), 125 (MD), 250 (HD) mg/kg/day BIA 2-093
Species/strain: Hsd: Sprague Dawley Rats
Number/sex/group: 25
Route, formulation, volume, and infusion rate: Oral gavage, 0.5% Hydroxypropylmethylcellulose, 5 ml/kg
Dosing solution analyses/Drug stability and homogeneity: Samples analyzed on Day 1 were within the specification of 10% of the nominal concentration. However, samples from week 6 were found to be out of specification. Reserve samples demonstrated that this deviation from specification was due to lack of homogeneity of the first set of Week 6 samples.

Satellite groups used for toxicokinetics: None
Study design: Males were dosed for 28 days before mating, during mating and for at least two weeks after the mating period. Females were dosed for 14 days prior to mating, during mating and until GD6.

Results:
Mortality and Clinical Signs: One MDF and two HDFs were euthanized *in extremis* on Days 14, 12 and 25, respectively. A third HDF was found dead on Day 15. These rats all had a distended GI tract containing a firm, dry green material. This finding is consistent with the firm and distended abdomens observed in all HDFs. No marked clinical signs were observed in LD animals.

Body weight: Consistent alterations in BW gain were not observed in males and in females in the premating phase that were exposed to BIA 2-093. However, MDFs and HDFs did exhibit a dose dependent decrease in BW gain from GD 0 to GD 13 (15% and 17%, respectively), when compared to controls.

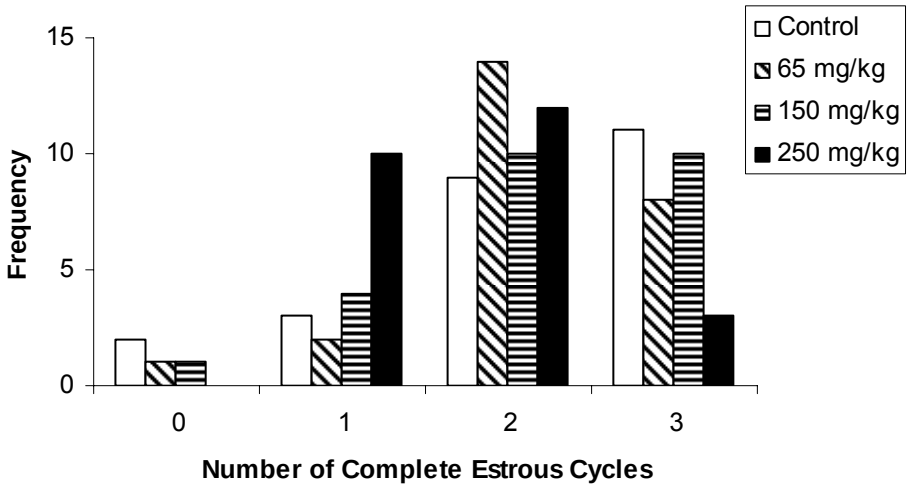
Food consumption: Overall, no test article related effect on food consumption was observed.

Water Consumption: A significant increase in maternal water consumption was observed in all BIA 2-093 exposed groups during GD 0-13, when compared to controls (9%, 15%, 25%, in LD, MD and HD, respectively).

Necropsy: Overall, no dose-dependent gross pathological abnormalities were observed in BIA 2-093 exposed males and females. However, a consistent observation in animals euthanized *in extremis* (1 female 125 mg/kg and 2 females 250 mg/kg) or found dead (1 female 250 mg/kg and 1 male 65 mg/kg) was distended GI tract containing unidentified firm, green material.

As with previous Studies, the Sponsor did not assay sperm viability, sperm counts or spermatogenesis via histopathological methods. Testes weight was not altered in BIA 2-093 exposed males.

Fertility parameters: The Sponsor reports that the group mean number of estrous cycles was decreased in rats exposed to 250 mg/kg/day BIA 2-093 for 14 days prior to mating. The frequency histogram for this data is presented below (Reviewer's Figure 9.1). Decreased (not statistically significant) numbers of corpora lutea, implantations and live fetuses were observed in the 250 mg/kg dose group. BIA 2-093 did not affect the number of days to first mating, the number of sperm plugs, the Copulation Index, the Fertility Index, the % pre-implantation loss, or the number of early embryo deaths.



Reviewer's Figure 9.1: BIA 2-093 Decreases the Number of Estrous Cycles in Female Rats. Above is a frequency histogram for the number of estrous cycles in each dose group. Females were exposed for 14 days to BIA 2-093 prior to mating. Females exposed to 250 mg/kg BIA 2-093 exhibited a decrease in the number of estrous cycles observed over the course of 15 days of observation. This decrease bordered on, but did not reach, statistical significance (Kruskal-Wallis One Way ANOVA, Corrected H=7.37, p=0.09).

9.2 Embryonic Fetal development

9.2.1 Mouse

Study title: **Oral (Gavage) Developmental Toxicity Study in the Mouse**

Key study findings:

- **The design and execution of this pivotal study were adequate.**
- **Skeletal abnormalities (incomplete ossification) made up the majority of developmental findings observed in fetuses from dams exposed to 650 mg/kg BIA 2-093.**
- **Major malformations that were not observed in the control group but were observed in fetuses from BIA 2-093 exposed dams were exencephaly and cleft palate.**
- **Dams exposed to 650 and 350 mg/kg BIA 2-093 exhibited unsteady gait, decreased activity, slow breathing, flat posture and partially closed eyes.**
- **Analysis of plasma samples demonstrates exposure to BIA 2-194, BIA 2-195 and OXC, metabolites of BIA 2-093.**

Study no.: 093-834
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 4/2/2007
GLP compliance: Yes
QA statement: Yes

Drug, lot #, and % purity: BIA 2-093, 25570-1-3, Purity = 99.3%

Methods

Doses: 0, 150 (LD), 350 (MD), 650 (HD) mg/kg

Species/strain: Crl:CD-1 (ICR) BR VAF/PLUS

Number/sex/group: 30 time mated females per group

Route, formulation, volume, and infusion rate: Oral gavage, 0.5% Hydroxypropylmethylcellulose, 5 ml/kg

Dosing solution analyses/Drug stability and homogeneity: Samples of dosing solutions were determined to be between 98% and 102% of the theoretical concentration.

Satellite groups used for toxicokinetics: Not performed.

Study design: Timed mated female mice were exposed to vehicle control or BIA 2-093 daily from GD6 to GD15, inclusive. Animals were euthanized on GD 18.

Results

Mortality (dams): No BIA 2-093 related mortalities occurred.

Clinical signs (dams): Dams exposed to 350 and 650 mg/kg BIA 2-093 exhibited unsteady gait, decreased activity, slow breathing, flat posture and partially closed eyes. These observations occurred intermittently between Days 7-14. No clinical signs were observed in mice exposed to 150 mg/kg.

Body weight (dams): No effect was observed on BW gain or total BW in LDFs. Mice exposed to 350 mg/kg exhibited a 66% decrease in BW gain on day one of dosing but subsequent values were similar to controls. Mice exposed to 650 mg/kg had reduced absolute BW (11%) when compared to controls, which persisted for the duration of the study.

Food consumption (dams): Food intake was depressed by 17%, when compared to controls, in HDFs from GD 6-12.

Toxicokinetics: BIA 2-093 and its metabolites were measured in mouse plasma samples collected on GD15, four hours after dosing.

Text Table 1 - Group mean plasma concentrations (ng/mL) of BIA 2-093 and its metabolites

Analyte	Group 1 Control	Group 2 150 mg/kg/day	Group 3 350 mg/kg/day	Group 4 650 mg/kg/day
BIA 2-093	BLQ	BLQ	BLQ	BLQ
BIA 2-194	BLQ	6227 ± 2941	12620 ± 7627	31450 ± 29085
BIA 2-195	BLQ	18.4	141 ± 43	106 ± 33
Oxcarbazepine	BLQ	2870 ± 1461	4837 ± 3156	8917 ± 4109

Sponsor's Summary Table 1: Toxicokinetics

Necropsy and Cesarean Section: Four out of 30 (13.3%) of the HDFs were found not to be pregnant at the end of the study. Of the dams that were pregnant in this dose group (26/30), only 23 (88%) had live fetuses at the time of euthanasia. Consistent with the decrease in the number of dams pregnant at the end of the study, the number of females with implantations at the end of

the study was reduced in HDFs (25/30) but not in any other dose group (Sponsor's Table 2). The number of total implants was decreased in the HD group (due to the lower number of HDF), but the number of implants per female was unchanged in all dose groups (Sponsor's Table 6).

Group	:	1	2	3	4
Test article	:	Control		BIA 2-093	
Dose (mg/kg/day)	:	0	150	350	650

Number of females:	Group 1		Group 2		Group 3		Group 4	
In group	30		30		30		30	
Not pregnant (%)	0	(0.0)	2	(6.7)	1	(3.3)	4	(13.3)
Died/killed	0		1		0		0	
Survived to scheduled kill	0		1		1		4	
Pregnant (%)	30	(100.0)	28	(93.3)	29	(96.7)	26	(86.7)
Died/killed/aborted	0		0		0		2	
with total resorptions	2		0		0		2	
with live foetuses at scheduled kill	28		28		29		23	
Littered	2		3		3		0	

Note: Littered females have been included in the totals for 'with live foetuses at scheduled kill'.

Sponsor's Table 2: Pregnancy in Mice Dosed with Eslicarbazepine Acetate

Group	:	1	2	3	4
Test article	:	Control		BIA 2-093	
Dose (mg/kg/day)	:	0	150	350	650

	Group 1	Group 2	Group 3	Group 4
Number of females with implantations at scheduled kill	30	28	29	25
Number of implantations	389	393	377	330
Mean number per female#	13.0	14.0	13.0	13.2
Standard deviation	3.5	2.5	3.3	4.5
Number of early embryo/foetal deaths	28	22	23	20
Number of late embryo/foetal deaths	6	3	3	3
Number of dead foetuses	0	0	0	2
Mean % post-implantation loss#	13.6	6.8	7.4	14.7
Number of live foetuses	355	368a	351	305
Mean number per female#	11.8	13.1	12.1	12.2
Standard deviation	3.9	2.7	3.4	4.8
Mean % of implantations	86.4	93.2	92.6	85.3

# = statistically analysed
a = includes Animal 31 (2F) which had 2 empty implants, sex of pups unknown

Sponsor's Table 6: Implantations in Pregnant Mice Dosed with Eslicarbazepine Acetate

Offspring: The number of females with live fetuses at the time of euthanasia was decreased exclusively in HDFs (number of females with live fetus/ number of pregnant females= 28/30, 28/28, 29/29, 23/26; C, LD, MD, HD, respectively). The mean fetal weight was also decreased by 12% in the HD group. No change in fetal weight was observed in the other dose groups. Fetuses from HDFs had a 7.6 fold higher incidence of major abnormalities and a 24% increase, relative to control, in the number of fetuses with variations (Sponsor's Table 8). A dose-dependent increase in the number of litters exhibiting fetuses with major abnormalities was also observed (2/28, 5/28, 6/29, 9/23, C, LD, MD, HD, respectively).

Table 8 - Foetal examination : summary of group mean data

(Page 1 of 1)

Group	: 1	2	3	4
Test article	: Control		BIA 2-093	
Dose (mg/kg/day)	: 0	150	350	650

	Group 1	Group 2	Group 3	Group 4
Combined examination (external/visceral/skeletal)				
Total number of litters examined	28	28	29	23
Total number of foetuses examined	355	366	351	307
Number with major abnormalities	2	8	7	12
Mean % of foetuses examined#	0.5	2.4	2.0	3.8**
Number of litters affected	2	5	6	9
Number with minor abnormalities	44	37	36	43
Mean % of foetuses examined#	13.2	10.7	10.9	15.0
Number of litters affected	20	20	20	18
Number with variations	145	129	134	153
Mean % of foetuses examined#	40.7	34.7	37.5	50.5*
Number of litters affected	28	28	29	23

= statistically analysed *=p<0.05 **=p<0.01 ***=p<0.001

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Sponsor's Table 8: Fetal Abnormalities

There were rare instances of major malformations in fetuses from the BIA 2-093 exposed dams that did not occur in the control group (Sponsor's Table 9). Specifically, exencephaly was observed to occur in fetuses from 3 out of 29 litters from the MD group and 1 out of 23 litters from the HD group. The incidence of these findings was 0/355 in controls, 0/366 in the LD group, 3/351 in the MD group and 1/307 in the HD group. Cleft palate also occurred in fetuses from BIA 2-093 exposed dams but not in the fetuses of control dams (Litters: 0/28 in controls, 3/28 in LD, 2/29 in MD, 3/23 in HD). All litters that exhibited this effect from the MD and HD groups had one fetus per litter with cleft palate. The LD had one litter with 3/11 fetuses with cleft palate, another with 2/12 fetuses with cleft palate and the third with only one fetus in the litter with cleft palate (Reviewer's Table 9.1). The incidence of this finding in the historical controls is 0-0.9%.

Dose (mg/kg)	Dam #	Fetus #	Malformation
350	74	L4	**Exencephaly**, open eye, malformed frontal skull, parietal skull absent, interparietal skull absent, occipital skull absent, malformed squamosal skull, 25 presacral vertebrae, asymmetric insertion of the pelvic girdle
350	87	L4	**Exencephaly**, malformed frontal skull, parietal skull absent, interparietal skull absent, occipital skull absent, malformed squamosal skull, Non-ossified ventral plate of the cerebral vertebrae, <6 central caudal vertebrae, cervical rib

350	88	R5	**Exencephaly**, open eye, malformed frontal skull, parietal skull absent, interparietal skull absent, occipital skull bifid, malformed squamosal skull, 14 thoracic vertebrae, 5 lumbar vertebrae, extra 14th rib
650	103	R5	**Exencephaly**, malformed frontal skull, parietal skull absent, interparietal skull absent, occipital skull absent, Non-ossified cerebral vertebrae, 14 thoracic vertebrae, 5 lumbar vertebrae, <6 caudal vertebrae, extra 14th rib, non ossified hind limb astragalus, non-ossified hindlimb phalange
150	35	L1	**Cleft palate**, cleft palantine skull
150	35	L2	**Cleft palate**, cleft palantine skull
150	35	R5	**Cleft palate**, cleft palantine skull, <6 centra caudal vertebrae
150	56	L3	**Cleft Palate**, Cleft palatine skull, non-ossified cervical vertebrae centra, <6 caudal vertebrae, non-ossified hindlimb astragalus
150	56	L4	**Cleft Palate**, Cleft palatine skull, misshapen or misaligned bilobed ossification of the sternum, non-ossified cervical vertebrae centra, <6 caudal vertebrae centra, <3 neural arches in caudal vertebrae, cervical rib, incomplete ossification of the 5th sternebra, non ossified hindlimb astragalus
150	58	L6	**Cleft Palate**, cleft palatine skull, incomplete ossification of 4th sternebra, incomplete ossification of ischium, incomplete ossification of pubis, left sided umbilical artery, incomplete ossification parietal skull, non-ossified cervical vertebrae, <6 caudal vertebrae, cervical rib, incomplete ossification of 5th sternebra, incomplete ossification 6th sternebra, non-ossified phalange, non-ossified astragalus, non-ossified hindlimb phalange
350	61	R5	**Cleft Palate**, cleft palatine skull, 27 presacral vertebrae, 14 thoracic vertebrae, <3 neural arches in caudal vertebrae, extra 14th rib
350	73	L4	**Cleft Palate**, cleft palatine skull, abnormal hind paw flexure

650	94	R6	**Cleft Palate**, cleft palatine skull, one or more ossified sternum centers, asymmetric insertion of entire pelvic girdle, 14 thoracic vertebrae, 5 lumbar vertebrae, <6 caudal vertebrae, extra 14th rib
650	105	L3	**Cleft Palate**, cleft palatine skull, incomplete ossification of the frontal skull, misshapen neural arch in cervical vertebrae
650	119	R3	**Cleft Palate**, cleft palatine skull, non-ossified cervical vertebrae, <6 caudal vertebrae, non-ossified hindlimb astragalus

Reviewer's Table 9.1: Major Abnormalities, Minor Abnormalities and Variants in Fetuses exhibiting Cleft Palate or Exencephaly.

Table 9 - Examination of foetuses : group mean data

Number of foetuses affected (group mean percent)

(Page 1 of 9)

Group	:	1	2	3	4
Test article	:	Control		BIA 2-093	
Dose (mg/kg/day)	:	0	150	350	650

External examination of foetuses

Key	Finding	Type	Group 1		Group 2		Group 3		Group 4	
	Total number of foetuses examined		355		366		351		307	
	Total number of litters examined		28		28		29		23	
	Head									
A	eye- uni- or bilateral: open	major	1	(0.2)	0	(0.0)	2	(0.5)	1	(0.3)
	Brain									
B	exencephaly	major	0	(0.0)	0	(0.0)	3	(0.8)	1	(0.3)
	Oral cavity									
C	palate: cleft	major	0	(0.0)	6	(1.9)	2	(0.5)	3	(1.0)
	Forelimb									
1	forepaw- uni- or bilateral: abnormal flexure	minor	0	(0.0)	1	(0.2)	0	(0.0)	0	(0.0)
	Hindlimb									
2	hindpaw- uni- or bilateral: abnormal flexure	minor	3	(0.8)	3	(0.8)	2	(0.5)	1	(0.4)

All findings statistically analysed

Sponsor's Table 9 (excerpted): Fetal Abnormalities

Skeletal abnormalities made up the majority of developmental findings observed in fetuses from HDFs (Sponsor's Table 9 (excerpted); Study 093-834 and Sponsor's Text Table 2; Study 093-834). Incomplete ossification of the occipital skull, unossified cervical vertebra, decreased number of centra and neural arches in the caudal vertebra, an increase in rib abnormalities, altered ossification of sternebra, and unossified phalanges in the hind limbs and forelimbs were present in fetuses from the HD group.

Overall, the Sponsor does not provide a conclusion regarding the potential for teratogenicity of eslicarbazepine acetate. The Sponsor does acknowledge the skeletal variants that occur in the HD group.

Text Table 2 – Group mean percentage of minor and variant abnormalities that achieved statistical significance.

Skeletal Findings	Background Data (%)	Group 1 (%)	Group 2 (%)	Group 3 (%)	Group 4 (%)
Occipital : incomplete ossification (v)	1.4 – 16.7	5.6	3.6	5.8	15.3***
Cervical centra, one or more : not ossified (v)	1.9 – 5.8	4.9	3.4	5.6	**35.3*** **
Cervical centra, one or more : incomplete ossification (v)	0.0	0.0	0.0	0.0	**2.7***
Cervical : additional ossification centre: present (m)	0.0	0.0	0.0	0.0	**1.4***
Thoracic : one or more centra: offset (m)	0.0	0.0	0.0	0.0	**1.1***
Thoracic : one or more centra: hemicentric (m)	0.0	0.0	0.0	0.0	**1.1***
Thoracic centra, one or more : incomplete ossification (m)	0.0 – 0.5	0.7	0.0	0.0	**3.7***
Caudal centra ≤ 6 (v)	15.5 – 45.5	16.8	16.6	21.5	**61.4*** **
Caudal neural arches ≤ 3 (v)	2.1 – 15.3	3.5	5.4	2.4	**16.6*** **
Rib, one or more fused (m)	0.0	0.0	0.0	0.0	**2.0***
Rib, one or more : floating (m)	0.0 – 0.5	0.0	0.4	**1.4**	**1.8***
5th sternebra : not ossified (v)	0.0	0.0	0.0	0.0	**2.3****
5th sternebra: incomplete ossification (v)	2.1 – 7.8	2.4	4.5	2.7	**9.2****
Sternebra, 5th or 6th : bilobed, bipartite, mishapen, misaligned (v)	0.0 – 5.5	0.0	0.0	0.5	1.3*
Forelimb, one or more phalange : not ossified (v)	0.0 – 38.8	1.5	1.7	1.2	18.2***
Astragulus, uni- or bilateral : not ossified (v)	7.6 – 41.4	12.6	10.6	13.0	**51.8*** **
Hindlimb, one or more phalange : not ossified (v)	0.7 – 34.4	1.5	0.6	0.0	14.6***

All findings statistically analysed. *=p<0.05, **=p<0.01, ***=p<0.001
(m) = minor abnormality, (v) = variant
Figures in bold fall ouside background data

Sponsor's Summary Table: Minor and Variant Skeletal Abnormalities

9.2.2 Rat

Study title: **Oral (Gavage) Developmental Toxicity Study in the Rat**

Key study findings:

- **The design and execution of this pivotal study were adequate.**
- **Multiple foci of incomplete ossification were observed in the skull, vertebrae, sternum and pelvic girdle in the fetuses from the MD and HD groups.**
- **A sustained decrease in BW gain and food consumption was observed in the dams MD and HD groups.**
- **Fetuses in the MD and HD group also exhibited decreased body weight.**
- **Small but statistically significant decreases in the number of live fetuses/dam was observed in MDF and HDF.**

Study no.: 093-835
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 3/23/2001
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093; 0000012976; Purity= 99.35%
Methods

Doses: 0, 65 (LD), 125 (MD), 250 (HD) mg/kg
Species/strain: Hsd: Sprague Dawley SD rats
Number/sex/group: 24 time mated females.
Route, formulation, volume, and infusion rate: Oral gavage, 0.5% Hydroxypropylmethylcellulose, 5 ml/kg
Dosing solution analyses/Drug stability and homogeneity: Initial analysis of Day 1 dosing samples were outside of the accepted specifications. Duplicate samples were obtained and determined to be within 10% of the nominal concentration. Week 2 samples were within 10% of the nominal concentration.
Satellite groups used for toxicokinetics: TK analysis was not performed
Study design: Timed-mated female rats were dosed daily with BIA 2-093 from GD6-GD17, inclusive. Animals were euthanized on GD 20.

Results

Mortality (dams): One animal from the HD group was euthanized *in extremis* after the first dose and was replaced with another female. The Sponsor does not state if this death was dose related but does state that this animal had a firm distended stomach.

Clinical signs (dams): HDFs had slightly swollen abdomens during the whole dosing period. This clinical sign was also observed in previously described studies in which rats were dosed with BIA 2-093 by oral gavage.

Body weight (dams): A slight decrease in BW gain was initially observed in all BIA 2-093 dose groups when compared to controls. The decrease in body weight gain persisted until the end of the study in the MDFs (13%) and HDFs (36%).

Food consumption (dams): Food consumption was reduced in all dose groups throughout the dosing phase of the study (10%, 14%, 28% in LD, MD, HD, respectively).

Terminal and macroscopic evaluations: A slight but statistically significant decrease in the number of live fetuses per females was observed in the MD and HD groups when compared to controls (13.5, 13.0, 12.1, 12.4 pups/female in control, LD, MD and HD, respectively).

Offspring: A dose-dependent decrease in mean litter weight was observed in the MD (12%) and HD (16 %,) groups when compared to controls. This decrease in mean litter weight coincided with the decreased maternal BW gain observed in the MD and HD groups. Mean fetal weight was decreased by 9% in the HD group when compared to control.

Multiple foci of incomplete ossification were detected in the skull, vertebrae, sternum and pelvic girdle in the MD and HD group (Sponsor's Table 12; Study 093-835); however, BIA 2-093 did not cause major fetal abnormalities in the rat.

Table 12 - Skeletal Examination of foetuses : group mean data

Number of foetuses affected (group mean percent)

(Page 1 of 5)

Group	:	1	2	3	4
Treatment	:	Control		BIA 2-093	
Dosage (mg/kg/day)	:	0	65	125	250

Key	Finding	Type	Group 1#	Group 2#	Group 3#	Group 4#
	Total number of foetuses examined		162	155	145	142
	Total number of litters examined		23	23	23	22
	Skull					
d	interparietal: incomplete ossification	variant	3 (1.7)	5 (3.1)	14 (9.5) **	16 (11.3) ***
e	occipital: incomplete ossification	variant	3 (1.7)	3 (2.2)	9 (5.9) *	13 (9.1) **
	Thoracic vertebra					
g	one or more centra: incomplete ossification	variant	6 (3.7)	10 (6.5)	20 (13.7) **	31 (21.3) ***
	Caudal vertebra					
P	entire- bilateral: absent	major	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)
31	number of centra: <=2	minor	3 (1.8)	2 (1.2)	11 (7.6) *	20 (13.5) ***
	Sternum (continued)					
j	5th sternebra: not ossified	variant	8 (4.6)	3 (2.0)	8 (5.5)	17 (11.8) *
k	5th sternebra: incomplete ossification	variant	8 (4.8)	14 (9.3)	18 (12.3) *	26 (18.1) ***
m	6th sternebra: not ossified	variant	2 (1.1)	1 (0.6)	1 (0.7)	3 (2.4)
n	6th sternebra: incomplete ossification	variant	6 (3.6)	5 (3.1)	17 (11.3) **	23 (16.3) ***
	Pelvic girdle					
46	pubis- uni- or bilateral: incomplete ossification	minor	3 (1.6)	4 (2.7)	5 (3.2)	9 (6.2) *

Sponsor's Table 12 (excerpted): Fetal Skeleton Examination Exposed to BIA 2-093 *In Utero*.

9.2.3 Rabbit

9.2.3. A. Study title: Toxicokinetics and Metabolism Study in the Pregnant Rabbit

Key study findings:

- **BIA 2-093 is rapidly metabolized to the major metabolite, BIA 2-194, in the pregnant rabbit.**
- **Females exposed to 320 mg/kg BIA 2-093 exhibited decreased activity, unsteady gait, prostration, rapid breathing and loss of limb use from 0.75 to 5 hours after dosing.**

- **BW gain was depressed in the MD and HD groups beginning on GD9. This resulted in a terminal BW gain that was ~60-65% of Controls.**

Study no.: 093-517
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 1/26/2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, 25570-1-3, Purity= 99.3%
Methods

Doses: 0, 40 (LD), 160 (MD), 320 (HD) mg/kg/day
Species/strain: HsdlF:NWZ rabbits, timed-mated
Number/sex/group: 3 females/group (0 and 40 mg/kg) and 4 females/group (160 and 320 mg/kg)
Route, formulation, volume, and infusion rate: Oral gavage, 0.5% Hydroxypropylmethylcellulose, 2 ml/kg
Dosing solution analyses/Drug stability and homogeneity: All samples met the specifications of 85% to 115% of the theoretical concentration.
Study design: Timed-mated rabbits were dosed once daily from GD6-GD18.

Results
Mortality (does): None

Clinical signs (does): Clinical signs were not observed in the LDF and MDF. HDFs exhibited decreased activity, unsteady gait, prostration, rapid breathing and loss of limb use from 0.75 to 5 hours after dosing.

Body weight (does): BW gain was depressed in the MD and HD groups beginning on GD9. This resulted in a terminal BW gain that was ~60-65% of Controls.

Toxicokinetics: Plasma was analyzed from all animals in the study on GD6 (single dose) and GD18 (repeat dose). TK parameters are contained in the table below. BIA 2-093 plasma levels were below the limit of quantification in all dose groups at both sampling time points. BIA 2-194, BIA 2-195 and OXC were detected in the plasma of BIA 2-093 exposed rabbits.

Day	Dose (mg/kg/day)	Metabolite	C_{max} (ng/mL)	t_{max} @ (hr)	AUC_{0-t} (hr*ng/mL)	$t_{1/2}$ (hr)	R_o	R_{lin}
6	40	BIA 2-194	3810 (27.5)	2.02 (0.500-4.00)	15600 (25.8)	1.35 (27.1)[2]	NA	NA
		BIA 2-195	NC~	NC~	NC~	NC~	NA	NA
		Oxcarb	299 (22.9)	2.02 (2.02-4.00)	789 (57.9)	NC+	NA	NA
6	160	BIA 2-194	28700 (1.48)[2]	2.00 (2.00-2.00)[2]	95800 (26.6)[2]	1.31 (NC)[1]	NA	NA
		BIA 2-195	66.0 (24.6)[2]	2.00 (2.00-2.00)[2]	93.8 (165)[2]	NC+	NA	NA
		Oxcarb	1400 (16.2)[2]	2.00 (2.00-2.00)[2]	6050 (39.6)[2]	1.56 (NC)[1]	NA	NA
6	320	BIA 2-194	55500 (65.4)	3.00 (0.500-4.00)	246000 (40.4)	1.31 (15.6)[3]	NA	NA
		BIA 2-195	144 (42.5)	4.00 (2.00-4.00)	400 (71.8)	NC+	NA	NA
		Oxcarb	2710 (38.1)	4.00 (2.00-4.00)	14400 (23.3)	1.63 (10.6)[3]	NA	NA
18	40	BIA 2-194	2990 (60.7)	2.00 (0.520-2.02)	11400 (17.3)	1.49 (28.0)[2]	0.732 (41.5)	0.811 (52.4)[2]
		BIA 2-195	NC~	NC~	NC~	NC~	NC	NC
		Oxcarb	274 (74.3)	2.00 (2.00-2.02)	841 (72.1)	NC+	1.07 (44.6)	NC
18	160	BIA 2-194	25600 (21.5)[2]	2.00 (2.00-2.00)[2]	104000 (1.04)[2]	NC+	1.08 (27.7)[2]	1.31 (NC)[1]
		BIA 2-195	90.8 (26.3)[2]	2.00 (2.00-2.00)[2]	225 (16.6)[2]	NC+	2.39 (214)[2]	NC
		Oxcarb	2330 (22.6)[2]	2.00 (2.00-2.00)[2]	10600 (6.43)[2]	2.27 (NC)[1]	1.75 (32.6)[2]	2.09 (NC)[1]
18	320	BIA 2-194	69000 (32.1)	0.500 (0.500-4.00)	230000 (16.6)	2.90 (5.16)[2]	0.937 (56.6)	0.965 (71.0)[3]
		BIA 2-195	156 (30.2)	2.00 (2.00-4.00)	544 (45.7)	NC+	1.36 (52.2)	NC
		Oxcarb	3490 (32.7)	1.25 (0.500-4.00)	17700 (18.4)	2.05 (37.3)[3]	1.23 (25.6)	1.22 (31.2)[3]

@ = Median (range). Oxcarb = Oxcarbazepine. NA = Not applicable.
NC+ = Not calculated; a terminal mono-exponential decline could not be unambiguously identified.
NC~ = Not calculated; all plasma samples below the limit of quantification (< 50 ng/mL).
NC = Not calculated.
N = 3 for 40 mg/kg/day and 4 for 160 and 320 mg/kg/day; unless otherwise stated [N].

Sponsor's Table: Toxicokinetics Summary

9.2.3. B. Study title: **Oral (Gavage) Developmental Toxicity Dose Range Finding Study in the Rabbit**

Key study findings:

- **One female exposed to 320 mg/kg (HD) was found dead on GD12.**
- **No dose-dependent abnormalities were observed in fetuses from BIA 2-093 exposed dams.**

Study no.: 093-836
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 3/30/2001
GLP compliance: Yes

QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, 0000012976, Purity = 99.6%
Methods

Doses: 0, 40, 80, 160, 320 mg/kg/day
Species/strain: Timed-mated New Zealand White rabbits
Number/sex/group: 5
Route, formulation, volume, and infusion rate: Oral gavage, 0.5% Hydroxypropylmethylcellulose, 2 ml/kg
Dosing solution analyses/Drug stability and homogeneity: Samples of dosing solutions were outside the specifications of ± 10% of the nominal concentration and were, therefore, not homogeneous. Retained dosing samples were found to be within the prescribed specification of ± 10% of the nominal concentration.
Satellite groups used for toxicokinetics: Not performed
Study design: Timed-mated rabbits were dosed once daily with BIA 2-093 from GD6-GD18, inclusive. Animals were euthanized on GD 28.

Results

Mortality (does): One female exposed to 320 mg/kg was found dead on GD12. No reason for the death is provided by the Sponsor and is, therefore, assumed to be treatment-related.

Clinical signs (does): No clinical signs.

Body weight (does): There was no treatment effect on absolute BW or BW gain.

Food consumption (does): No statistically significant effect on food consumption was observed in any dose group.

Terminal and macroscopic evaluations: No BIA 2-093 related effects were observed on litter weight, fetal weight, fetal sex ratio, number of live fetuses per doe, number of corpora lutea or the number of implantations per female.

Offspring: One fetus in the 40 mg/kg group was a runt and exhibited acephaly. No abnormalities were observed in other dose groups.

9.2.3. C. Study title: Oral (Gavage) Developmental Toxicity Study in the Rabbit

Key study findings:

- **The design and execution of this pivotal study was adequate.**
- **All groups exposed to BIA 2-093 exhibited an increased number of fetuses with an increased number of presacral vertebrae when compared to controls. A NOEL was not determined.**
- **Irregular ridging of the palate was observed in fetuses from all groups exposed to BIA 2-093, but not in fetuses from the control group.**
- **A greater percentage of fetuses in the HD group (a maternally toxic dose) when compared to controls, exhibited incomplete ossification of the maxilla, non**

ossification of the humerus epiphyses, incomplete ossification of the phalanges on the forelimb and the hind limb.

- **Twenty five percent (5/20) of the females in the HD group and five percent (1/20) of the MD group were euthanized *in extremis* due to the presentation of severe clinical signs such as loss of movement in all limbs, hypoactivity, irregular breathing, lateral recumbence, impaired mobility and uncoordination**
- **From GD6 (the beginning of the dosing period) until GD15 for the MD group and GD 22 for the HD group, a consistent decrease in BW gain was observed.**
- **A decrease in female fetus BW, but not male fetus BW, was observed in the MD (7%) and HD (10%) groups.**

Study no.: 093-837
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 6/15/2001
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, 0000012976, Purity = 99.7%

Methods

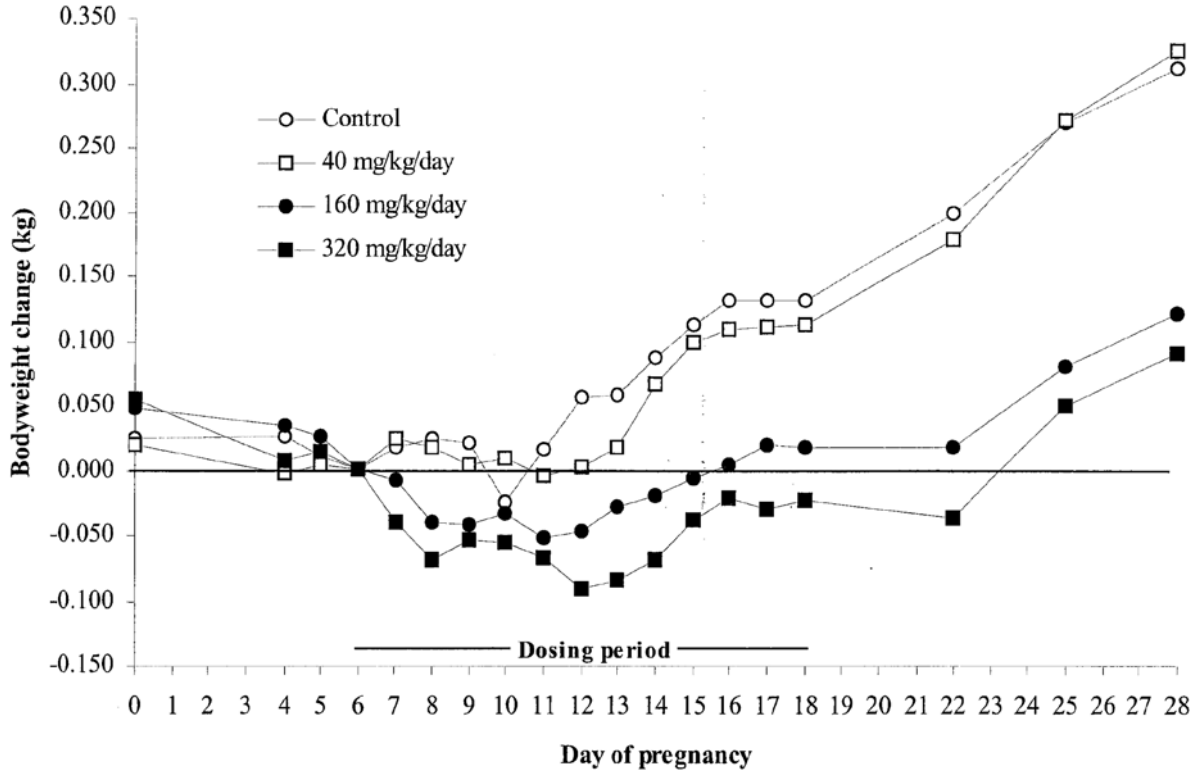
Doses: 0, 40 (LD), 160 (MD), 320 (HD) mg/kg
Species/strain: Timed-mated New Zealand White rabbit
Number/sex/group: 20
Route, formulation, volume, and infusion rate: Oral gavage, 0.5% Hydroxypropylmethylcellulose, 2 ml/kg
Dosing solution analyses/Drug stability and homogeneity: Dosing solutions were within the specification of 10% of the nominal concentration.
Satellite groups used for toxicokinetics: Not performed.
Study design: Timed-mated rabbits were dosed once daily with BIA 2-093 from GD6-GD18, inclusive. Animals were euthanized on GD 28.

Results

Mortality and Clinical Signs (does): 5 HDF and 1 MDF were euthanized *in extremis* at Day 12, 17 or 18 of pregnancy due to the presentation of severe clinical signs such as loss of movement in all limbs, hypoactivity, irregular breathing, lateral recumbence, impaired mobility and uncoordination. Additionally, one HDF was not pregnant at the end of the study and another aborted spontaneously before the end of the study. No clinical signs were observed in the LD group. In the surviving MDF and HDF, reduced or abnormal feces was the main clinical sign observed.

Body weight (does): A consistent negative BW change was observed in MDF from GD6 (the beginning of dosing) to GD15 (~50 g) and in HDF from GD6-GD22 (~50-75g). There was no effect of treatment on the LD group (Sponsor's Figure 1). At the conclusion of the study on Day 28, there was no treatment related effect on mean absolute BW (4.2 kg, 4.2 kg, 4.0 kg, 4.0 kg; C, LD, MD, HD, respectively).

Figure 1 - Group mean maternal bodyweight change (kg)



Sponsor's Figure 1: Group mean maternal bodyweight change

Food consumption (does): A decrease in maternal food consumption was observed in the MD and HD groups during a portion of the dosing period (GD6 to GD12 and GD20 to GD22). This coincided with the period of negative BW change observed in Sponsor's Figure 1.

Terminal and macroscopic evaluations: A decrease in female fetus BW, but not male fetus BW, was observed in the MD (7%) and HD (10%) group (Sponsor's Table 5). No test article related effects were observed in the number of corpora lutea per female, number of implantations per female, number of embryo deaths, post-implantation loss, number of live fetuses per female or fetus sex ratio (Sponsor's Table 6). There were no dose-related abnormalities observed in the gross pathology analysis of the does.

Table 5 - Group mean uterine / implantation data

(Page 1 of 1)

Group	:	1	2	3	4
Treatment	:	Control		BIA 2-093	
Dosage (mg/kg/day)	:	0	40	160	320

	Group 1	Group 2	Group 3	Group 4
Number of females with implantations at scheduled kill	18	20	17	13
Number of corpora lutea	183	194	172	127
Mean number per female	10.2	9.7	10.1	9.8
Standard deviation	2.1	1.7	1.8	1.5
Number of implantations	165	169	155	115
Mean number per female	9.2	8.5	9.1	8.8
Standard deviation	1.8	2.3	2.0	2.3
Mean % pre-implantation loss	8.9	12.7	9.7	10.4
Number of early embryo/foetal deaths	17	6	12	13
Number of late embryo/foetal deaths	18	8	20	5
Number of dead foetuses	0	0	0	0
Mean % post-implantation loss	20.0	7.9	20.0	13.2
Number of live foetuses	130	155	123	97
Mean number per female	7.2	7.8	7.2	7.5
Standard deviation	2.1	2.1	2.7	2.0
Mean % of implantations	80.0	92.1	80.0	80.6

Sponsor's Table 5: Implantation Data

Table 6 - Group mean litter weights (g) / foetal data

(Page 1 of 1)

Group	:	1	2	3	4
Treatment	:	Control		BIA 2-093	
Dosage (mg/kg/day)	:	0	40	160	320

	Group 1	Group 2	Group 3	Group 4
Number of females with live foetuses at scheduled kill	18	20	16	13
Number of live foetuses	130	155	123	97
Mean number per female	7.2	7.8	7.7	7.5
Standard deviation	2.1	2.1	2.0	2.0
Number of male foetuses	63	69	60	40
Number of female foetuses	67	86	63	57
Mean % male foetuses	46.4	47.7	49.0	43.8
Mean litter weight	275.1	307.7	280.6	269.4
Standard deviation	69.0	81.7	77.5	72.1
Mean foetal weight	38.8	39.9	36.6	36.3
Standard deviation	4.3	2.8	4.0	3.9
Mean foetal weight - males only	38.5	40.7	37.9	36.8
Standard deviation	4.9	3.4	2.9	4.1
Mean foetal weight - females only	38.6	40.0	35.9*	34.9*
Standard deviation	4.9	3.4	4.0	4.5
Mean placental weight	4.36	4.53	4.08	4.22
Standard deviation	0.75	1.09	0.40	0.85

* = significantly different from Controls, p<0.05 (Williams test)

Sponsor's Table 6: Litter and Fetal Weight

Offspring: An increase in the number of minor visceral abnormalities was observed in the HD group (0% of controls vs. 4.9 % of HD fetuses). The number of litters affected was also increased in the HD when compared to controls (Sponsor's Table 7; Reviewer's Table 9.2). The rate of minor skeletal abnormalities was also increased in fetus from the HD group (20.5% in controls vs. 38.2% in HD fetuses; Sponsor's Table 7). The number of litters with minor skeletal abnormalities did not differ from controls.

Table 7 - Examination of foetuses : summary of group mean data

(Page 2 of 6)

Group	:	1	2	3	4
Treatment	:	Control		BIA 2-093	
Dosage (mg/kg/day)	:	0	40	160	320

	Group 1	Group 2	Group 3	Group 4
Fresh visceral examination				
Total number of litters examined	18	20	16	13
Total number of foetuses examined	130	155	123	97
Number with major abnormalities	2	3	0	2
Mean % of foetuses examined	1.5	3.9	0.0	2.7
Number of litters affected	2	3	0	2
Number with minor abnormalities	0	2	0	4*
Mean % of foetuses examined	0.0	1.7	0.0	4.9
Number of litters affected	0	2	0	4
Number with variations	21	21	9	11
Mean % of foetuses examined	13.8	14.7	6.8	10.3
Number of litters affected	9	11	6	7

* = significantly different from Controls, P<0.05 (Williams test)

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Table 7 - Examination of foetuses : summary of group mean data

(Page 5 of 6)

Group	:	1	2	3	4
Treatment	:	Control		BIA 2-093	
Dosage (mg/kg/day)	:	0	40	160	320

	Group 1	Group 2	Group 3	Group 4
Skeletal examination				
Total number of litters examined	18	20	16	13
Total number of foetuses examined	130	155	123	97
Number with major abnormalities	1	1	1	3
Mean % of foetuses examined	0.5	0.6	0.7	2.6
Number of litters affected	1	1	1	3
Number with minor abnormalities	31	36	37	39**
Mean % of foetuses examined	20.5	23.0	31.0	38.2
Number of litters affected	12	18	13	11
Number with variations	127	149	121	96
Mean % of foetuses examined	97.9	96.8	98.1	99.0
Number of litters affected	18	20	16	13

** = significantly different from Controls, P<0.01 (Fishers test and Cochran-Armitage trend test)

Sponsor's Table 7: Summary of fetal visceral and skeletal abnormalities

Dose (mg/kg)	Dam #	Fetus #	Malformation
0	4	L4	**Cystic Dilation of Cerebral Hemisphere**
	7	L2	**Cystic Dilation of Cerebellum**
	11	L1	**Pulmonary Valvular Atresia**
	13	R6	**Malformed Cervical Vertebra (neural arch)**
	19	L1	**Interrupted Aortic Arch**
40	23	R6	**Enlarged Subarachnoid Space**
	28	L1	**Pulmonary Valvular Atresia**
	33	R1	**Spina Bifida, Bifid Sacral Vertebra, Bifid Caudal Vertebra**
	34	L1	**Pulmonary Valvular Atresia**
	38	R5	**Pulmonary Valvular Atresia**
	39	R1	**Cystic Dilation of Cerebellum**
160	43	L2	**Cystic Dilation of Cerebellum**
	58	R1	**Fused Sternum**
	59	R6	**Cystic Dilation of Cerebellum**
320	64	L3	**Cystic Dilation of Cerebellum**
	65	L6	**Bowed Forelimb**
	71	L4	**Pulmonary Valvular Atresia**
	72	R3	**Gastroschisis, Forelimb Ectrodactyly, Brachydactyly, Hindlimb Ectrodactyly, Malformed Premaxilla, Malformed Nasal Skull, Short Digit on Forelimb, Absent Digit on Forelimb, Absent Phalange on Forelimb**
	73	L1 R1	**Fused Thoracic Vertebra** **Pulmonary Valvular Atresia**

Reviewer's Table 9.2: Listing of Major Malformations Identified in Sponsor's Table 7.

Examination of the heads of fetuses with Bouin's stain demonstrated an increase in irregular ridging of the palate (0/61, 1/73, 1/57, 2/47 fetuses in control, LD, MD and HD, respectively). This finding was observed in 0/18, 1/20 (5%), 1/ 20 (5%) and 2/13 (15%) C, LD, MD, and HD litters, respectively. The occurrence of this finding in historical controls (Appendix 11 of the study report) is 0-3.3%. Therefore, this abnormality is considered to be drug-related.

Skeletal abnormalities were observed in the fetuses from dams exposed to BIA 2-093 (Sponsor's Table 12). Specifically, all dose groups exhibited a higher percentage of fetuses with an increased number of presacral vertebrae when compared to controls. This was mainly due to an increase in the number of thoracic vertebrae. Also, a greater percentage of fetuses in the HD group (a maternally toxic dose), when compared to controls, exhibited incomplete ossification of the maxilla, non-ossification of the humerus epiphyses, incomplete ossification of the phalanges on the forelimb and the hind limb.

Overall, the Sponsor concluded that the minor abnormalities and variations that occurred in the MD and HD were dose-related.

Group	:	1	2	3	4
Treatment	:	Control		BIA 2-093	
Dosage (mg/kg/day)	:	0	40	160	320

Key Finding	Type	Group 1	Group 2	Group 3	Group 4
Total number of foetuses examined		69	82	66	50
Total number of litters examined		18	20	16	13
Skull					
c maxilla- uni- or bilateral: incomplete ossification	variant	20 (30.3)	16 (23.2)	28 (44.4)	24 (43.7)*
Vertebra					
17 number of presacral vertebrae: 27	minor	6 (4.3)	25 (17.0)**	17 (14.7)**	12 (13.0)
Forelimb					
q epiphyses: not ossified	variant	40 (28.0)	32 (21.1)	46 (35.9)	23 (24.9)
r proximal or distal epiphyses of humerus only: not ossified	variant	52 (41.3)	69 (44.7)	49 (38.9)	51 (51.4)*
s one or more phalange: incomplete ossification	variant	45 (35.2)	55 (36.8)	40 (33.4)	45 (46.5)*
Hindlimb					
v one or more phalange: incomplete ossification	variant	5 (3.6)	13 (10.2)	16 (12.8)**	20 (19.1)***

Sponsor's Table 12 (excerpted): Minor and Variant Skeletal Abnormalities. *=p<0.05, **=p<0.01, ***=p<0.001.

9.3 Prenatal and postnatal development

9.3.1. Mouse

9.3.1. A. Study title: **Oral (Gavage) Pre and Post-natal Developmental Toxicity Dose Range Finding Study in the Mouse**

Key study findings:

- **All pregnant dams exposed to 500 mg/kg BIA 2-093 exhibited unsteady gait, flat posture, palpebral closure, slow breathing and decreased activity on the first day of dosing. All dams exposed to 650 mg/kg/day exhibited these clinical signs during the entire dosing period (GD6-GD12).**
- **Dams exposed to 500 mg/kg/day BIA 2-093 exhibited pale foci in the liver when euthanized at PN6.**
- **There was a slight decrease in BW from PN4 to PN7 in male and female pups born to dams exposed to 500 mg/kg when compared to controls from PN4 to PN7**.

Study no.: 093-838
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 1/15/2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, 25570-1-3, Purity = 99.3%
Methods

Doses: 0, 500, 600 mg/kg/day
Species/strain: CD1 mouse
Number/sex/group: 10 mated females
Route, formulation, volume, and infusion rate: Oral gavage, 0.5% hydroxypropylmethylcellulose, 5ml/kg
Dosing solution analyses/Drug stability and homogeneity: Dosing solutions were determined to be homogeneous since the deviation between top, middle and bottom sampling was less than 10%. Also, all samples met the specification of ± 15% of the nominal concentration.
Satellite groups used for toxicokinetics: None
Study design: Timed-mated female mice were dosed with 500 mg/kg once a day from GD 6 to postnatal day (PN) 6. Females exposed to 650 mg/kg/day were dosed once a day from GD 6 to GD12; females were euthanized on GD13.

Results
F_0 in-life: No mortality was observed in any of the animals exposed to BIA 2-093. All pregnant dams exposed to 500 mg/kg BIA 2-093 exhibited unsteady gait, flat posture, palpebral closure, slow breathing and decreased activity on the first day of dosing. On the second day of dosing, all pregnant dams exposed to 500 mg/kg only exhibited unsteady gait and decreased activity. Select animals from this dose group continued to exhibit decreased activity (6/10), unsteady gait (6/10), flat posture (6/10), slow breathing (4/10) and palpebral closure (2/10) during gestation. All dams

exposed to 650 mg/kg/day exhibited unsteady gait, flat posture, slow breathing, and decreased activity during the entire dosing period (GD6-GD12).

A slight reduction in BW gain was observed in the dams exposed to 500 and 650 mg/kg on GD 6 and GD7. However, absolute body weights at the end of gestation were not different in either dose group when compared to controls. BW and BW gain did not differ between the dams exposed to 500 mg/kg and controls during lactation. However, food consumption by dams receiving 500 mg/kg was decreased by 16%, relative to controls, during PN 4-7.

No statistically significant differences were observed between control dams and dams exposed to 500 mg/kg in gestation index, mean number of live pups born, pup sex ratio, mean live birth index, mean viability index, mean lactation index and cumulative survival index. The number of live embryos in dams exposed to 650 mg/kg was lower when compared to controls (10.6 live embryos in 650 mg/kg group vs. 12.9 pups in the control group); there was no control group euthanized on GD 13.

F_0 necropsy: At necropsy, no treatment effect on the number of implantation scars was observed. However, dams exposed to 500 mg/kg/day BIA 2-093 exhibited pale foci in the liver when euthanized at PN6. No overall abnormalities in the gross pathological examination of dams exposed to 650 mg/kg were observed on GD 13.

F_1 physical development: There was a slight decrease in BW of male (~12-13%) and female (~12-14%) pups born to dams exposed to 500 mg/kg when compared to controls from PN4 to PN7. There were no findings during the litter necropsy that were considered to be related to maternal treatment with BIA 2-093.

9.3.1. B. Study title: **Oral (Gavage) Pre and Post-Natal Development Toxicity Study in the Mouse**

Key study findings:

- **As observed in previous studies, all dams exposed to MD (350 mg/kg) and HD (650 mg/kg) exhibited decreased activity, unsteady gait, flat posture and slow breathing.**
- **Decreased BW relative to controls was observed in HD dams during gestation. BW relative to controls and food intake was decreased in MD and HD dams during lactation.**
- **Duration of gestation was increased by one-half day in the HD group.**
- **BW in the F_1 generation was decreased relative to controls for the duration of lactation in the MD and HD groups.**
- **Eye and ear opening was delayed in pups from the HD group.**
- **The Sponsor used the E-maze to assess the impact of BIA 2-093 on learning and memory in F1 offspring. This cognitive test is considered to have a low sensitivity for assessing the impact of test articles on learning and memory.**
- **The time to preputial separation and vaginal perforation was delayed in a dose-dependent fashion in the MD and HD groups.**
- **Exposure of dams to BIA 2-093 did not impact the reproductive functioning of the offspring.**

- **Table 26 of this report lists dydrogesterone (1, 10, 100 mg/kg bid), not BIA 2-093, as the test article. An explanation for this heading was requested of the Sponsor by the Division on 2/23/10.**

Study no.: 093-839
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 3/26/2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, 25570-1-3, Purity = 99.3%

Methods

Doses: 0, 150 (LD), 350 (MD), 650 (HD) mg/kg/day
Species/strain: Crl:CD-1 (ICR) BR VAF/PLUS mice
Number/sex/group: F_0 generation= 30 timed mated females; F_1 = 22 sex/group
Route, formulation, volume, and infusion rate: Oral gavage, 0.5% hydroxypropylmethylcellulose, 5 ml/kg
Dosing solution analyses/Drug stability and homogeneity: Dosing solutions were determined to be homogeneous since the deviation between top, middle and bottom sampling was less than 2.5%. Also, all samples met the specification of ± 10% of the nominal concentration
Satellite groups used for toxicokinetics: Not performed
Study design: Timed-mated females (F_0) were dosed once a day from GD6 until PN20, inclusive. F_1 pups were examined pre and post weaning and pups from the same dose group were mated at ten weeks of age. Pregnant dams from the F_1 generation were euthanized on GD13 to assess pregnancy parameters and F_2 fetuses.

Results

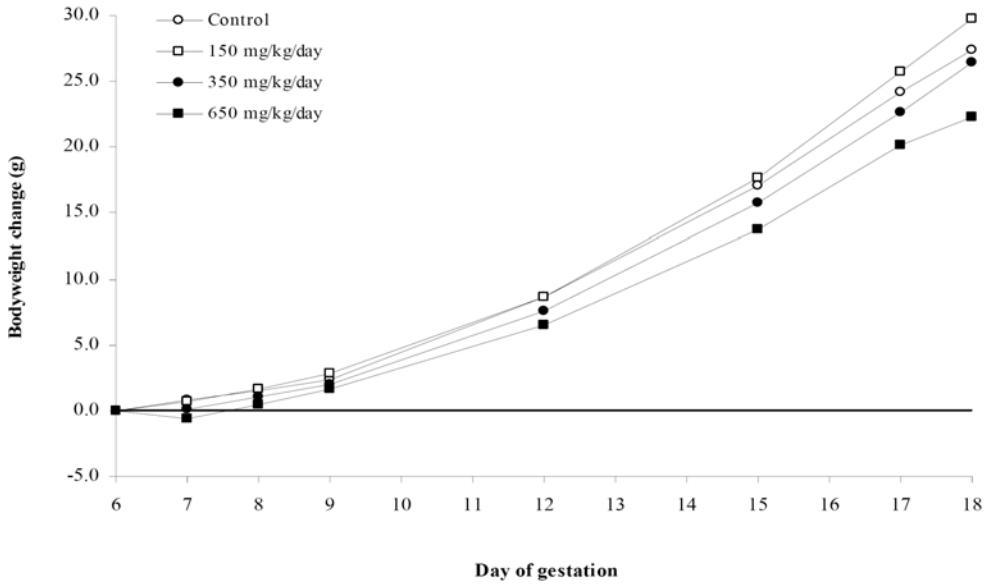
F_0 in-life:

Mortalities: One MD female convulsed on PN 21 of the study and died just before scheduled euthanasia. This effect may be related to exposure to BIA 2-093.

Clinical Signs: As observed in previous studies, all MD and HD dams exhibited decreased activity, unsteady gait, flat posture and slow breathing on Days 6 and 7 of gestation. These clinical signs lasted for at least 5.5 hrs and were observed intermittently during the gestational period of the study in both groups. During the lactation (postnatal) phase of the study, unsteady gait was observed intermittently in the HD group over the course of the first six days of lactation (26/26 females) and decreased activity was observed in the dams from the HD group (9/26 dams). No clinical signs were observed in the LD group.

Body Weight: No alteration in maternal BW and BW gain was observed in dams exposed to 150 mg/kg/day (Sponsor's Figure 1). HDFs exhibited a 17% decrease in BW gain throughout the gestational period, when compared to controls. BW gain was decreased in the MD (~23%) and HD (~29%) dams during lactation.

Figure 1 - Group mean maternal bodyweight change (g) : F0 generation females - gestation



Food Consumption: Food consumption was decreased by 10-20% during most of the gestational period in HD dams (GD 6-12 and GD 15-18) and intermittently in MD dams (GD 9-12 and GD 15-18). Overall food consumption was decreased in the MD (7-10%) and HD (11-17%) dams during lactation.

Gestation and Parturition: A statistically significant increase in the mean duration of gestation by almost one half day occurred in dams from the HD group (19.6 days in controls vs. 20 days in HDFs). No effect of BIA 2-093 was evident on gestation index, number of pups born per litter, live birth index, lactation index, cumulative survival index, or sex ratio.

F_0 necropsy: No abnormal gross pathology findings were observed in dams exposed to BIA 2-093.

F_1 physical development:

Body Weight: A dose dependent decrease in BW and BW gain (relative to controls), which persisted for the entire lactation period, was observed in pups born to dams from the MD and HD groups (Sponsor's Table 11; 11% and 19%, respectively). Pups born to dams exposed to 150 mg/kg *in utero* did not exhibit altered BW or BW gain. Additionally, both F_1 males and females from the HD group exhibited a slight decrease (6% and 14%, respectively) in mean BW at the time of mating.

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Table 11 - Group mean pup bodyweights and bodyweight gains (g) : F1 generation litters

(Page 1 of 2)

Group	:	1	2	3	4
Test article	:	Control		BIA 2-093	
Dose (mg/kg/day)	:	0	150	350	650

Males

Bodyweight (Day of age)	Group 1	2	3	4
N	27	22	25	26
1#	1.8 ± 0.2	1.8 ± 0.1	1.7 ± 0.1**	1.7 ± 0.1***
4#	2.7 ± 0.4	2.7 ± 0.2 (21)	2.6 ± 0.2 (24)*	2.4 ± 0.3***
7#	4.7 ± 0.5	4.8 ± 0.3 (21)	4.4 ± 0.4 (24)**	4.0 ± 0.6***
14#	8.8 ± 0.9	8.5 ± 0.5 (21)	7.8 ± 0.5 (24)***	7.4 ± 1.5***
21#	13.8 ± 1.6	13.8 ± 0.7 (21)	12.4 ± 1.0 (23)***	11.5 ± 1.7 (25)***
Bodyweight gain (g)# (Day 1 to 21)	12.0 ± 1.5	12.0 ± 0.6 (21)	10.7 ± 1.0 (23)***	9.8 ± 1.6 (25)***

N = number of litters in mean
# - statistically analysed *=p<0.05 **=p<0.01 ***=p<0.001
() = N, where different from the original

Table 11 - Group mean pup bodyweights and bodyweight gains (g) : F1 generation litters

(Page 2 of 2)

Group	:	1	2	3	4
Test article	:	Control		BIA 2-093	
Dose (mg/kg/day)	:	0	150	350	650

Females

Bodyweight (Day of age)	Group 1	2	3	4
N	27	22	25	26
1#	1.7 ± 0.2	1.7 ± 0.1	1.6 ± 0.1*	1.6 ± 0.1**
4#	2.7 ± 0.4	2.6 ± 0.2 (21)	2.4 ± 0.2**	2.3 ± 0.3***
7#	4.6 ± 0.5	4.7 ± 0.3 (21)	4.2 ± 0.4**	3.9 ± 0.6***
14#	8.7 ± 1.0	8.4 ± 0.6 (21)	7.7 ± 0.6***	7.3 ± 1.4***
21#	13.6 ± 1.5	13.3 ± 0.8 (21)	12.2 ± 1.1 (24)***	11.1 ± 1.6 (25)***
Bodyweight gain (g)# (Day 1 to 21)	11.8 ± 1.4	11.6 ± 0.7 (21)	10.6 ± 1.1 (24)***	9.5 ± 1.6 (25)***

N = number of litters in mean
# - statistically analysed *=p<0.05 **=p<0.01 ***=p<0.001
() = N, where different from the original

Sponsor's Table 11: F1 Generation Body Weight and Body Weight Gain

Developmental Observations: Pups from the HD group exhibited a delay in development characterized by a reduced number of pups with open ears on PN 4 (13.9% in controls vs. 4.0 % in HD) and open eyes on PN 15 (67.7% in controls vs. 53.8% in HD). The timing of the assessment of these reflexes is consistent with that of previous studies conducted in mice [7,8].

F_1 behavioral evaluation: Exposure to BIA 2-093 during gestation and lactation did not alter the development of pupillary light reflex in F_1 pups when assessed on postnatal days 33-37.

Furthermore, the Preyer reflex (a measure of hearing acuity) and Rotarod performance (a measure of balance and coordination) were not altered in F_1 mice from any dose group when measured on postnatal days 33-37. E-maze performance (a measure of cognitive function) was not altered in male pups exposed to BIA 2-093 during development. Although a test of cognitive ability, a simple test such as the E-maze is not considered to be sufficiently complex for a comprehensive assessment of the impact of test articles on learning and memory [9,10]. Finally, the Reviewer notes that in the original submission, Table 26 incorrectly lists the test article as dydrogesterone at 1, 10, 100 mg/kg bid. An explanation for this was requested of the Sponsor on 2/23/10.

F_1 reproduction: Impaired sexual development was observed in F_1 offspring from the MD and HD groups. Specifically, a delay in preputial separation (2 days in HD) and vaginal perforation (1.4 days in MD and 2.4 days in HD) was observed. The mean number of days taken to mate was statistically significantly decreased in MD and HD (4.0, 3.8, 2.7, 3.0 days in control, LD, MD and HD, respectively). There was no effect of BIA 2-093 exposure on the mean number of sperm plugs per animal, the copulation index and the fertility index. These results suggest that BIA 2-093 exposure of F_0 dams does not impact the fertility of F_1 animals.

F_2 findings: The number of resorptions, the number of implantations in F_1 dams, the number of early embryo deaths, and the number of live embryos per pregnant F_1 female were not altered in groups where the F_0 dams were dosed with BIA 2-093. These results suggest no affect of BIA 2-093 exposure in utero and indirectly during lactation on embryo-fetal development in F_1 dams

9.3.2. Rat

Study title: **Oral (Gavage) Pre- and Post-natal Developmental Toxicity Study in the Rat**

Key study findings:

- **The design and execution of this pivotal study was adequate.**
- **Several dams from each dose group were euthanized *in extremis* due to the exhibition of severe clinical signs such as unsteady gait, decreased activity, piloerection and hypothermia.**
- **Dams from the HD group exhibited sustained decreases in BW gain and food intake during gestation and lactation. Water consumption was increased in all dose groups during gestation.**
- **Gestation duration was lengthened slightly in the HD group.**
- **Viability index trended towards a dose-dependent decrease in the MD and HD group.**
- **Cumulative survival index was decreased (67%) in the HD group.**
- **BIA 2-093 may decrease milk production in rats since a dose dependent increase in the number of pups with empty stomachs and persistently decreased pup BW was observed in the MD and HD groups.**
- **No alterations in balance, cognition, Preyer reflex and pupillary reflex were detected in pups exposed to BIA 2-093 during gestation and lactation. However, the Sponsor used the E-maze to assess the impact of BIA 2-093 on learning and memory**

in F1 offspring. This cognitive test is considered to have a low sensitivity for assessing the impact of test articles on learning and memory.

- **Sexual maturity was delayed in pups from the HD group. Preputial separation and vaginal perforation was delayed in pups from the HD group.**
- **Besides a decrease in the number of corpora lutea in pregnant F_1 females from all dose groups, no other reproductive abnormalities were observed in offspring from dams exposed to BIA 2-093.**

Study no.: 093-840
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 4/27/2001
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BIA 2-093, 0000012976, Purity = 100%

Methods

Doses: 0, 65 (LD), 125 (MD), 250 (HD) mg/kg
Species/strain: Timed-mated Hsd: Sprague Dawley SD rats
Number/sex/group: 25
Route, formulation, volume, and infusion rate: Oral gavage, 0.5% Hydroxypropylmethylcellulose, 5 ml/kg
Dosing solution analyses/Drug stability and homogeneity: With the exception of the 13 mg/ml solution, dosing solutions were determined to be homogeneous since the deviation between top, middle and bottom sampling was less than 10%. A reserve sample for the 13 mg/ml solution was analyzed and was found to be within specification. Also, all samples met the specification of ± 10% of the nominal concentration.
Satellite groups used for toxicokinetics: Not done
Study design: Timed-mated females (F_0) were dosed once a day from GD6 until PN20, inclusive. F_1 pups were examined pre and post weaning and pups from the same dose group were mated at ten weeks of age. Pregnant dams from the F_1 generation were euthanized on GD13 to assess pregnancy parameters and F_2 fetuses.

Results

F_0 in-life:

Mortality and Clinical Signs: 3 LD dams (GD 9, GD 10, PN 7), 1 MD dam (GD 7) and 2 HD (PN 4, GD 23) dams which were euthanized *in extremis*. These dams all exhibited abnormal breathing, piloerection, hypoactivity, swollen abdomen, hypothermia, hunched posture and unsteady gait. Piloerection and swollen abdomen was the main clinical signs observed in MD and HD dams. These clinical signs are consistent with what was observed in previous studies and are considered treatment-related.

Body Weight: Absolute BW was consistently decreased throughout gestation by 6-11% of controls in dams from the HD group. A slight decrease in absolute BW of 3-5% of control occurred in LD and MD dams from GD 6-GD 9. In comparison with control dams, absolute BW was decreased throughout the lactation period by 6-9% in the HD dams.

Food consumption: Food intake was decreased by 20-52% in HD dams throughout the gestational period. A slight decrease (~16-27%) in food intake occurred in LD and MD dams

between GD 6-9, but returned to normal for the remaining period of gestation. During lactation, food intake was decreased by 8% in MD and HD groups.

Water consumption: Water consumption was increased in all dose groups (LD= 10-12%, MD= 20-25%, HD= 8-12%) when compared to controls from GD 6-GD 20. During the first week of lactation, water consumption was increased in MD (13%) and HD (6%) dams.

Gestation and Parturition: Gestation index, mean number of pups per litter, live birth index, lactation index and sex ration were not affected by BIA 2-093 exposure. However, the mean duration of gestation was increased slightly (0.4 days), but significantly, in HD dams. Mean cumulative survival index was significantly decreased (69.7%) in the HD group and viability index was decreased in MD and HD (92.6% and 85.5%, respectively; Sponsor's Table 8)

Table 8 - Group mean pregnancy and litter data : F0 generation females and F1 generation litters

(Page 1 of 1)

Group	:	1	2	3	4
Treatment	:	Control		BIA 2-093	
Dosage (mg/kg/day)	:	0	65	125	250

Group	Number littered (N)	Gestation index	Mean duration of gestation (days) ± S.D.	Mean duration of parturition (minutes) ± S.D.	Mean number of pups born ± S.D.	Mean live birth index	Mean viability index	Mean lactation index	Mean cumulative survival index	Sex ratio at birth
1	25	96.0	22.0 ± 0.5	108.2 ± 44.5(17)	11.4 ± 3.1	92.1	98.2(24)	98.4(24)	89.2	51.8 : 48.2
2	25	100.0	22.2 ± 0.6	154.1 ± 109.1(17)	11.9 ± 1.7	87.7	96.3(24)	100.0(22)	81.1(24)	46.6 : 53.4
3	25	100.0	22.2 ± 0.4	140.0 ± 47.4(13)	11.6 ± 2.0	94.2	92.6+	99.5	87.7	46.1 : 53.9
4	24	100.0	22.4 ± 0.5**	134.2 ± 81.8(18)	11.0 ± 2.5	81.7	85.5+	97.7(22)	69.7(23)+	46.2 : 53.8
Analysis of variance			NS	NS	NS	NS	NS	NS	NS	

N = number of animals in mean
() = N, where different from the original
+ = significantly different from the control, p<0.05, Shirleys' test
** = significantly different from the control, p<0.01, Williams' test
NS = not significant

Sponsor's Table 8: Pregnancy and Litter Data

F_0 necropsy: No abnormal gross pathology findings were observed in dams exposed to BIA 2-093. There was no difference among dose groups in the number of implantation scars.

F_1 physical development: Righting reflex, eye opening, startle response, ear opening and pupillary light reflex were similar to controls in all pups from BIA 2-093 exposed dams. A 13% decrease in BW gain was observed from PN 0-21 in the male pups born to HD dams. Female pups born to MD and HD dams exhibited decreased BW (6% and 10% respectively) during the same time period. The decrease in BW gain persisted into adulthood (up to PN 42 in females and PN 70 in males). During necropsy of F_1 generation pups, a dose-dependent increase in pups with no milk in their stomach was observed (4, 6, 13, and 22 in control, LD, MD and HD, respectively). When expressed as number of litters containing pups with no milk in the stomach, this measure was also increased when compared to control (Control =2 litters, LD= 6 litter, MD= 6 litters, HD= 7 litters). There were no other treatment-related abnormalities observed during necropsy.

F_1 behavioral evaluation: Overall, the F_1 generation offspring that had been exposed to BIA 2-093 during gestation and indirectly during lactation did not exhibit any alteration in cognitive performance as tested in the E-maze, auditory acuity as tested in the Preyer reflex test, and balance as tested in the Rotarod test. The exception to this was the F_1 offspring from the HD group exhibited a slight decrease in Rotarod time when compared to controls (93 seconds vs. 135 seconds, respectively).

F_1 reproduction: Sexual development was delayed in males from the F_1 generation born to dams from the HD group. The number of days until preputial separation was increased by 1.5 days in this group. There was a slight (not statistically significant) increase of 0.7 days for time to vaginal perforation (Control= 35.5 days vs. HD F_1 offspring= 36.2 days) in female F1 offspring from the HD group. There were no BIA 2-093 related alterations in time to mating, number of copulation plugs, fertility index, copulation index, number of implantations per pregnant F_1 female, pre-implantation loss, number of early embryo/fetal deaths, or the number of live embryos per pregnant female. However, the number of corpora lutea per F_1 female was decreased in all dose groups (17.6, 16.2, 15.6, and 16.3 in control, LD, MD and HD, respectively).

10. Overall integrated summary and safety evaluation:

Eslicarbazepine acetate (BIA 2-093) is a third generation, anti-epileptic pro-drug which is metabolized *in vivo* in humans, mice, dogs and rabbits to the major active metabolite (> 10% of drug-related exposure), S-licarbazepine (BIA 2-194), and two minor metabolites (< 10% of drug-related exposure), R-licarbazepine (BIA 2-195) and oxcarbazepine (OXC); the later being the major metabolite in rats. The conversion of BIA 2-093 to BIA 2-194 is mainly independent of CYP450 activity. The metabolites widely and rapidly distribute throughout the body, especially to the kidneys, liver, pancreas, salivary glands, thyroid, adrenal medulla, brain and to suckling pups via the milk of dams exposed to BIA 2-093. Elimination is mainly via the urine in mice and dogs. All three metabolites are antagonists of voltage-gated calcium channels (T-type) and inactivated, voltage-gated sodium channels; with OXC demonstrating the greatest potency at these targets (i.e., IC_{50} for calcium channels: 345, 337 and 135 μM for BIA 2-194, BIA 2-195 and OXC, respectively; IC_{50} for sodium channels: 562, 530 and 172 μM for BIA 2-194, BIA 2-195 and OXC, respectively). Possibly due to the ability of its metabolites to antagonize the voltage-gated sodium and calcium channels, BIA 2-093 was protective in various animal models of chemically and electrically-induced seizure. Although not as potent as CBZ and OXC in preventing bicuculine, picrotoxin and 4-aminopyridine-induced seizures, BIA 2-093 did inhibit the generation of seizures in animals exposed to these seizurogenic compounds and was more potent than OXC and CBZ at preventing metrazole-induced seizures. Overall, the pharmacokinetics and pharmacodynamics of BIA 2-093 in animal models suggest that BIA 2-093 may be useful as an antiepileptic medication in humans.

Toxicology studies were performed in rats, mice, rabbits and dogs, with mice, rabbits and dogs being the most relevant due to the similarity to humans in terms of their metabolism of BIA 2-093. In rats, however, the Sponsor demonstrates that BIA 2-093 and the major human metabolite, BIA 2-194, are predominately metabolized to OXC, a minor human metabolite. As a result, the toxicities identified in rats dosed with BIA 2-093 (e.g., increased incidence of chronic progressive nephropathy and diuresis) are difficult to interpret with regards to the safe human use of eslicarbazepine acetate. Therefore, the results of the toxicology studies performed in rats will not be discussed in this integrated summary. Along with relevant species (mouse and dog) being chosen by the Sponsor for the general toxicology assessment of BIA 2-093, the pivotal study conducted in the non-rodent species was of sufficient length (1 year) to support the safety of a chronic use medication. The longest general toxicology repeat-dose study in a relevant rodent model was a 13 week repeat-dose mouse study. However, the two-year mouse carcinogenicity study is of sufficient dosing duration to cover the maximum period of chronic dosing recommended in ICH M3(R2). Plasma exposure to BIA 2-194, the main human metabolite, was adequate in the pivotal studies performed in the relevant rodent model (Plasma BIA 2-194 AUC_{0-24hr} at MRHD= 336,147 ng*hr/ml; 13 week mouse study BIA 2-194 AUC_{0-24hr} = 494,377 ng*hr/ml). Although the plasma levels of the main human metabolite in the non-rodent study did not exceed the plasma levels observed at the MRHD (1 year beagle dog study AUC_{0-24hr} = 102,748 ng*hr/ml), the highest dose employed in the pivotal, non-rodent study was determined to be the MTD.

The general, repeat-dose toxicology studies performed in mice and dogs identified the liver, CNS, blood, heart, gastrointestinal system and kidneys as target organs. In addition, reproductive and developmental toxicology studies conducted in rabbits and mice and a mouse carcinogenicity study demonstrate that BIA 2-093 is a teratogen and a carcinogen. The relevant

findings in these studies are discussed below in relation to the safe use of eslicarbazepine acetate in humans.

In mice and dogs dosed chronically with BIA 2-093 by oral administration, liver weights were increased. However, only mice exhibited histopathological correlates such as centrilobular hepatocellular hypertrophy. Beginning at 4 weeks of repeat dosing, mice exhibited an increase in liver weight at all dose levels tested. Centrilobular hypertrophy, however, was only reported in mice dosed with BIA 2-093 for at least 13 weeks. In the 13 week study, a NOEL for increased liver weight and centrilobular hypertrophy could not be determined since this finding was present at the lowest dose tested. In dogs, liver weight was increased after chronic exposure to BIA 2-093 but in the absence of any histopathological alterations in the liver. Overall, the finding of increased liver weight and centrilobular hypertrophy is a common pharmacodynamic property of potent inducers of CYP 450 enzymes, a known pharmacodynamic property of BIA 2-093.

Adverse Effect	No Effect Level (NOEL)	MRHD	Safety Margin
"Seizure-like" activity			
Dog (3 Month)	C_{max}= 8,716 ng/ml	C_{max}= 22,957 ng/ml	0.4
Mouse (104 Week)	C_{max}= 30,125 ng/ml	C_{max}= 22,957 ng/ml	1.3
Increased APTT			
Dog (12 Month)	$AUC_{0\text{-}24hr}$= 34,126	$AUC_{0\text{-}24hr}$= 336,147	< 0.1*
GI Hypomotility			
Mouse (Single Dose)	150 mg/m^2	740 mg/m^2	< 0.2*
Teratogenicity			
Mouse (Embryo-Fetal)	450 mg/m^2	740 mg/m^2	< 0.6*
Rabbit (Embryo-Fetal)	480 mg/m^2	740 mg/m^2	< 0.7*
Impairment of Fertility			
Mouse (Fertility)	450 mg/m^2	740 mg/m^2	< 0.6*
Carcinogenicity			
Mouse (104 Week)	300 mg/m^2	740 mg/m^2	< 0.4

*Reviewer's Table 10.1: Summary Table of Safety Margins for Adverse Effects Observed in Nonclinical Studies. The study from which the safety margin is calculated is given in parenthesis. Cmax and $AUC_{0\text{-}24hr}$ represent plasma levels of BIA 2-194 in animals (NOEL) or humans (MRHD). Doses provided on the basis of mg/m^2 are based on the NOEL for nonclinical studies or the MRHD of 1200 mg/day in a 60 kg human. Safety margins are calculated as animal NOEL/MRHD. MRHD= Maximum Recommended Human Dose. *= No NOEL was determined for this adverse effect and the plasma level or dose represents the lowest dose employed in the nonclinical study.*

In repeat dose studies performed in dogs, clinical signs described by the Sponsor to be "seizure-like" (Study 093-817, Section 3.3. page 13) were observed. Although the Sponsor never characterizes these symptoms as distinct seizure episodes, the type and progression of these clinical signs are considered by the Reviewer to be suggestive of seizure manifestation. Beginning in the three month dog study (Study # 093-817), "seizure-like" symptoms were described by the Sponsor. These clinical signs began as early as 1.5 hours after dosing and lasted for up to 6 hours. The progression of symptoms consisted of a transition from reduced activity

(lethargy, drowsiness, subdued behavior) to disturbances in locomotor activity that progressed to muscular rigidity. As muscular rigidity was resolving, the dog usually continued to show decreased activity. However, the progression to muscular rigidity was not always preceded by drowsiness. Depending on the study, the proportion of dogs exhibiting these clinical signs ranged from 20-80% (n=5 per dose group) in the 3 month study to 25-50% (n=4 per dose group) in the 6 month study and 25-100% (n=4 per dose group) in the 12 month study. The safety margins, based on the NOEL mean plasma C_{max} for BIA 2-005 in male and female dogs and the C_{max} at the MRHD, for "seizure-like" symptoms are 0.4 (Dog= 8,716 ng/ml; Human= 22,957 ng/ml) in the 3 month dog study, 0.8 (Dog= 17,523 ng/ml; Human= 22,957 ng/ml) in the 6 month dog study and 1.3 (Dog= 30,811 ng/ml; Human= 22,957 ng/ml) in the 12 month dog study (Reviewer's Table 10.1). In the 13- and 104-week oral studies conducted in the mouse, manifestations of clinical signs that are considered by the Reviewer to be suggestive of seizure were also observed in the HD groups of these studies. However, it is important to mention that the Sponsor never classifies these as distinct seizure episodes. The safety margins, based on the NOEL mean plasma C_{max} for BIA 2-005 in male and female mice and the C_{max} at the MRHD, for the clinical signs suggestive of seizure are 3.2 (Mouse= 72,796 ng/ml; Human= 22,957 ng/ml) in the 13 week mouse study and 1.3 (Mouse= 30,125 ng/ml; Human= 22,957 ng/ml) in the 104 week mouse study. Overall, it is evident from the chronic dog and mouse studies that there is little to no safety margin for the manifestation of clinical signs suggestive of seizure at the MRHD.

The activated partial thromboplastin time (APTT) was elevated in the absence of an effect on prothrombin time (PT) and coagulation in all dog studies conducted by the Sponsor. This hematology parameter was not assessed in any of the mouse studies making it impossible to determine if this effect was observed in more than one species that produce BIA 2-194 as the major circulating metabolite of BIA 2-093. The safety margin for this effect based on the mean plasma concentrations of BIA 2-194 at the NOEL in male and female dogs ($AUC_{0\text{-}24hr}$) and the plasma concentration in humans exposed to the MRHD is < 0.03 (No NOEL identified, Dog < 9,637 ng*hr/ml; Human 336,147 ng*hr/ml) in the 4 week study, 0.1 (Dog= 44,179 ng*hr/ml; Human 336,147 ng*hr/ml) in the 3 month study, 0.2 (Dog= 62,188 ng*hr/ml; Human 336,147 ng*hr/ml) in the 6 month study and <0.1 (No NOEL identified, Dog< 34,126 ng*hr/ml; Human 336,147 ng*hr/ml) in the 12 month study (Reviewer's Table 10.1). The Sponsor does not provide data to support a mechanism of action for the increased APTT in dogs but it has been established that prolongation of APTT in the presence of normal PT is associated with deficiencies in Factors VIII, XI, IX, XII, prekallikrein and kininogen [11]. As was observed in the dog studies performed by the Sponsor, clinical expression of a coagulopathy is seldom apparent when APTT is prolonged in the absence of alterations of other coagulation parameters such as PT. Although, elevation of APTT in the absence of a coagulopathy may occur in cases of hepatopathy [11], dogs exposed to BIA 2-093 that exhibited prolonged APTT, did not exhibit an overt hepatopathy. Therefore, while the extremely low SMs for BIA 2-093-associated prolongation of APTT and the lack of an identified mechanism of action suggest a clinical concern in humans exposed to BIA 2-093, it is important to remember that this findings did not occur in conjunction with a coagulopathy in BIA 2-093 exposed dogs. It is unclear then, if BIA 2-093 exposure would be associated with a coagulopathy in humans.

BIA 2-093 induced-QT/QTc shortening (17-18 msec) was observed in non-clinical studies performed by the Sponsor. In isolated canine cardiac Purkinje fibers, a robust and dose-dependent shortening of the time to complete repolarization was observed when the fibers were

perfused with BIA 2-093, suggesting the potential for eslicarbazepine acetate to cause shortened QT/QTc *in vivo*. Indeed, *in vivo* studies do demonstrate that dosing with BIA 2-093 causes QT/QTc shortening in dogs. This QT/QTc shortening in dogs exposed to 210 mg/kg BIA 2-093 occurred at plasma concentrations of 25,700-35,214 ng/ml BIA 2-194 which would represent a SM of 1.1-1.5 when compared to the C_{max} in humans exposed to the MRHD (22,957 ng/ml). Calculation of SMs based on $AUC_{0\text{-}24hr}$ is not possible for this effect since this parameter was not calculated in the dog study. Although the exact toxicological relevance of QT/QTc shortening is not completely characterized, a recent publication suggests that QT/QTc shortening of > 100 msec when compared to normal subjects may be a risk factor for refractory or idiopathic ventricular fibrillation [1]. This conclusion is based on the observation that humans with an inherited short QT syndrome (< 360 ms) are susceptible to fatal arrhythmias. A review by the CDER Interdisciplinary Review Team for QT Studies determined that eslicarbazepine acetate at doses of 1200 and 2400 mg does not significantly alter the QT/QTc interval in humans. Therefore, while the finding of QT shortening in dogs dosed with BIA 2-093 is interesting, a similar shortening does not occur in humans dosed with eslicarbazepine acetate.

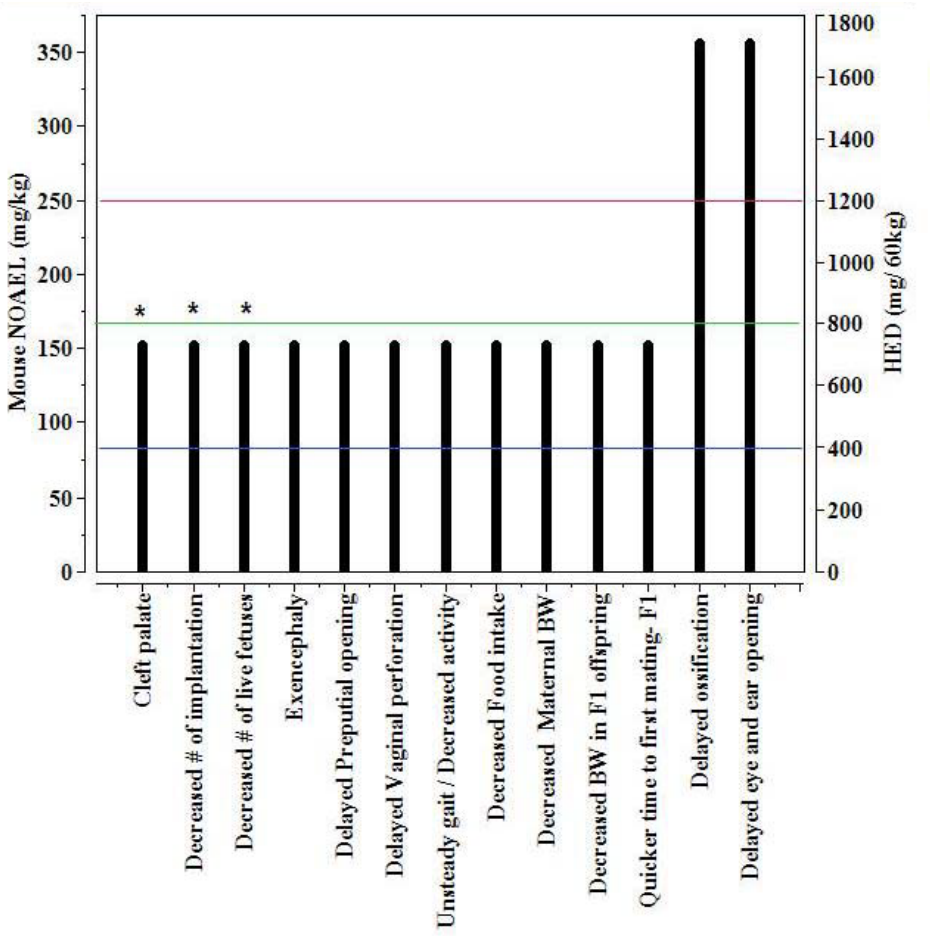
Gastrointestinal motility was decreased in mice exposed to oral doses of BIA 2-093. At the lowest dose tested (50 mg/kg) a 25% decrease in gastric motility was observed (Reviewer's Table 10.1; Safety margin <0.2). Although this finding in mice was robust, the relevance of this finding to the safe human use of eslicarbazepine acetate is unclear.

Decreased urine production in mice and decreased urinary electrolyte concentration in mice and dogs were observed in non-clinical studies of BIA 2-093, in the absence of significant histopathology findings in the kidney. A decrease in urine production (>62%) and urinary electrolytes (sodium, potassium and chloride) were observed in the forced urine production test in mice given single doses of ≥ 250 mg/kg BIA 2-093. Unfortunately, the Sponsor did not measure the plasma concentration of these electrolytes during this or any other mouse study submitted in NDA 22416, making it impossible to determine if the decrease in urinary electrolytes is mirrored by an expected increased retention of electrolytes in the plasma. Furthermore, although the Sponsor does characterize a decrease in urinary electrolytes in dogs similar to what was observed in mice, urinary volume is never reported. Therefore, it is not possible to determine if there is a concordant effect of BIA 2-093 in mice and dogs with regards to decreased urinary volume and urinary electrolytes. However, the Sponsor does measure plasma electrolyte concentrations from dogs expressing decreased urinary electrolytes and no overt alteration in plasma electrolytes is observed. It is interesting that hyponatremia, a finding in humans dosed with eslicarbazepine acetate (as described in the proposed labeling of STEDESA®), was not observed in the repeat-dose studies performed in this relevant animal species.

Regarding the reproductive and developmental toxicity studies, the most concerning adverse effect of BIA 2-093 is the occurrence of malformations in mice and rabbit fetuses exposed *in utero* to doses of BIA 2-093 that were not maternally toxic. A NOEL for teratogenicity could not be determined by the Sponsor due to the occurrence of palate malformations in mice (<150 mg/kg) and rabbits (<40 mg/kg) and extra presacral vertebrae in rabbits (<40 mg/kg) at the lowest dose tested. Although the frequency of palate malformations was low (cleft palate in mice= 0.5%-0.9%; irregular ridging of the palate in rabbits= 1.7%-4.1%) these malformations were not observed in control fetuses in either species. Furthermore, some rabbit fetuses in the control group exhibited an increased number of presacral vertebrae (~4%), but rabbit fetuses exposed to BIA 2-093 *in utero* exhibited a significant increase in the incidence of this

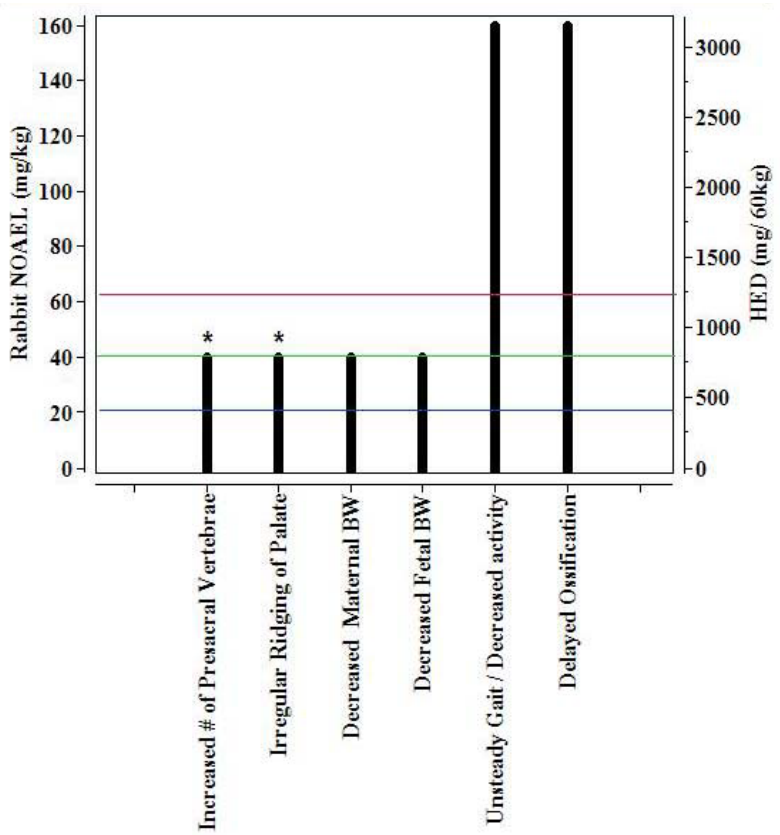
malformation (13-17%). Exencephaly was also observed in fetuses from mouse dams exposed to >150 mg/kg BIA 2-093, but not in controls (Exencephaly= 0.3-0.8%). Since many of these effects were observed at an animal dose (≤ 150 mg/kg for mice and ≤ 40 mg/kg in rabbits) that was lower than the MRHD (Reviewer's' Figure 10.1), it is possible that these malformations could occur in infants born to women on a chronic maintenance dose regimen of BIA 2-093 (safety margin for teratogenicity is < 0.6; Reviewer's Table 10.1). Other findings in the developmental toxicity studies, such as delayed ossification of the skeleton, occurred at maternally toxic doses (Reviewer's Figures 10.1-10.2). In summary, it is evident that there is no safety margin for BIA 2-093-related teratogenicity. However, teratogenicity has been reported in nonclinical studies of many types of FDA-approved antiepileptic medications, including those that have a similar structure to BIA 2-093 [12].

BIA 2-093 Effects on Mouse Reproduction



*Reviewer's Figure 10.1: Comparison of Reproductive Effects in Mice and Human Equivalent Dose (HED). *= LOAEL, no NOAEL determined for this effect. Blue Line= Lowest Human Dose (400 mg), Green Line= Proposed Human Maintenance Dose (800 mg), Red Line = Maximum Recommended Human Dose (1200 mg)*

BIA 2-093 Effects on Rabbit Reproduction



*Reviewer's Figure 10.2: Comparison of Reproductive Effects in Rabbits and Human Equivalent Dose (HED). *= LOAEL, no NOAEL determined for this effect. Blue Line= Lowest Human Dose (400 mg), Green Line= Proposed Human Maintenance Dose (800 mg), Red Line = Maximum Recommended Human Dose (1200 mg).*

Impairment of fertility was also observed in mice exposed to BIA 2-093. A decreased number of implantations and live fetuses were observed in mice at the lowest dose tested (150 mg/kg) and, therefore, a NOEL could not be determined for this effect (Reviewer's Figure 10.1; Reviewer's Table 10.1). Overall, these results suggest that decreased fertility in humans could be an outcome of exposure to BIA 2-093. The mechanism for decreased fertility in rodents exposed to BIA 2-093 in unclear.

The genotoxicity studies performed by the Sponsor, which were GLP compliant and consistent with current ICH guidelines, demonstrate that BIA 2-093 is not a mutagen in the bacterial reverse mutagenesis assay but is a weak mutagen in the *in vitro* mouse lymphoma assay and a clastogen in the CHO cell assay. The results of a computational toxicology analysis of BIA 2-093 and BIA 2-194 performed by the CDER/ ICSAS were not completely consistent with the results of these genotoxicity assays [13]. Specifically, the computational toxicology analysis of BIA 2-093 and BIA 2-194 was negative with respect to the *Salmonella* and *E. coli* mutagenesis assays. However, the computational analysis did not predict the clastogenic activity of BIA 2-093 observed in the *in vitro* chromosomal aberration assay in CHO cells.

Although the Sponsor performed the standard genotoxicity battery, a major deficit in the genetic toxicology studies described in NDA 22416 is the lack of sufficient characterization of the production of BIA 2-093 metabolites, specifically BIA 2-194 (S-licarbazepine), in the *in vitro* assays. Documentation of S-licarbazepine production in the *in vitro* assays was specifically requested by DNP in the IND 67,466 May Proceed Letter dated January 26, 2007 [14]; this information has never been received by the Division. Measurement of the extent of BIA 2-194 production in *in vitro* genotoxicity assays where rat hepatic S9 fractions were used as the biotransformation system is important because it has been demonstrated by the Sponsor that the rat is not a relevant species for human risk assessment due to its propensity to metabolize BIA 2-093 *in vivo* to OXC, a minor human metabolite. The Sponsor did, however, measure BIA 2-194 in an *in vitro* metabolism assay where BIA 2-093 was incubated in the presence and absence of rat liver microsomes. In this microsomal metabolism study (093-526), the Sponsor did demonstrate that BIA 2-194 is produced by rat liver microsomes but is detected at levels 20% lower than in the presence of human liver microsomes. Furthermore, OXC levels are 2 fold higher in rat liver microsomes when compared to human liver microsomes. It is important to remember that hepatic S9 fractions, which were used in the *in vitro* genotoxicity assays, contain both cytosolic and microsomal drug metabolizing enzymes. Therefore, the resulting metabolic profile obtained with rat hepatic S9 fractions may differ from the profile obtained with rat hepatic microsomes. Since the Sponsor did not measure the levels of BIA 2-194 produced in the presence of the rat liver S9 fraction, it is impossible for the Reviewer to determine if these assays contained sufficient levels of the major human metabolite, BIA 2-194. The Sponsor does attempt to address this issue by testing BIA 2-194 directly in the bacterial reverse mutation assay; BIA 2-194 was not a mutagen in the bacterial reverse mutation assay. The Sponsor does not, however, address this deficiency in the *in vitro* mammalian mutation assay or the *in vitro* chromosomal aberration assays, which exhibited equivocal and positive results, respectively, in the presence of BIA 2-093. Therefore, it is the Reviewer's conclusion that the *in vitro* genotoxicity assays conducted by the Sponsor in the presence of rat liver S9 fraction did not completely characterize the genotoxicity of the main human metabolite, BIA 2-194. However, from the studies provided by the Sponsor, it is obvious that BIA 2-093 is a weak mutagen in the *in vitro* mouse lymphoma assay and a clastogen with the ability to cause chromatid deletions, *in vitro*. Evidence of clastogenicity or mutagenicity was not observed in *in vivo* models of genetic toxicity (i.e., *in vivo* mouse micronucleus assay and *in vivo* unscheduled DNA synthesis assay) which concurred with the findings of the FDA ICSAS computational analysis [13].

The requirement for a rat carcinogenicity study was waived by DNP due to the inability to achieve adequate plasma levels of BIA 2-194 in rats since this species predominantly metabolizes BIA 2-093 and BIA 2-194 to OXC. The mouse carcinogenicity study, which was performed in a manner consistent with current ICH guidance, demonstrates that chronic exposure to BIA 2-093 over the course of two years causes hepatocellular carcinomas and hepatic adenomas in male and female mice. Based upon dose per body surface area, the doses used in the carcinogenicity study represent 0.4, 1.0 and 2.3 times the MRHD (Reviewer's Table 10.1). Therefore, it is clear that there is a narrow safety margin for the occurrence of liver neoplasia based upon the proposed MRHD for BIA 2-093 of 1200 mg. Labeling for STEDESA® should reflect the existence of a potential risk for hepatic neoplasia in users of BIA 2-093.

Concerning the finding of BIA 2-093-induced neoplasia, the Sponsor argues that the increased incidence of adenomas and carcinomas in mice has little relevance to human health. Specifically, the Sponsor argues that this increase in liver neoplasia occurs via a mechanism

similar to phenobarbital-induced carcinogenesis in rodents (Section 5.1.1. of the Toxicology Written Summary and in the Discussion Section of Study 093-830, respectively):

> *"A number of xenobiotics known to stimulate hepatic drug-metabolizing enzyme activity, liver growth, and multiplication of the smooth endoplasmic reticulum have also been found to induce liver tumors in rodents. Tumors are typically produced by long-term administration of doses that are associated with substantial increases in liver weight. Repeat-dose toxicology studies with SEP-0002093 in rodents had demonstrated substantial increases in liver weight and the induction of liver tumors was not unexpected in this 2-year study in mice."*

> *"Inducers of hepatomegaly such as Phenobarbitone produce hepatic tumors in the mouse following prolonged administration; however epidemiological evidence from the use of high doses over many years does not indicate any increased incidence of hepatic cancer in man (15). The mitogenic response associated with hepatomegaly appears to be absent in man and therefore the relevance of mouse hepatomegaly related liver tumors to human risk assessment is considered dubious."*

Although the mechanism of phenobarbital-mediated induction of liver tumors in rodents is well characterized to be of little relevance to humans [15], it is unclear if it is this mechanism that is involved in the increased incidence in liver adenomas and hepatocellular carcinomas in BIA 2-093-exposed mice because specific defining characteristics of this mechanism were not conclusively demonstrated by the Sponsor. Specifically, characteristics of the phenobarbital mechanism of hepatic neoplasia involve activation of a specific transcription factor resulting in induction of specific CYP450 enzymes, occurrence via a non-genotoxic mechanism, inhibition of apoptosis, proliferation of the smooth endoplasmic reticulum and hepatomegaly/ increased liver weight [15, 16].

Integral to the mechanism of phenobarbital induction of liver neoplasia is signaling via the constitutive androstane receptor (CAR), a transcription factor which controls the expression of many drug metabolizing enzymes [17, 18]. Of these enzymes, increased expression of members of the CYP2B family, such as 2B1 and 2B2, are indicative of increased activity via this phenobarbital-responsive transcription factor [15]. Other CYP450 isoforms induced via CAR are CYP 2A1, 3A1, 2C6, 2C7, 2C11 and 3A2 [15]. In the studies submitted to the Agency, the Sponsor does not demonstrate that the expression of any of these previously mentioned CYP 450 isoforms is increased in livers of mice exposed to BIA 2-093 or its metabolites. One study submitted by the Sponsor (093-638), in which mice were exposed to 30 mg/kg/day BIA 2-093 for 5 days, demonstrates an increased metabolism of substrates used to measure the activity of CYP 2A6, 2C9, 2C19, 2E1, and 3A4. Induction of CYP 2A6 and 3A4 has been reported after incubation of phenobarbital *in vitro* with human hepatocytes but the relationship to the induction of these specific isoforms and phenobarbital-induced hepatic neoplasia is unknown [19]. Therefore, the Sponsor has not sufficiently demonstrated the involvement of CAR in the hepatic neoplasia observed in mice exposed to BIA 2-093.

Another characteristic of phenobarbital-induced hepatic neoplasia is its occurrence via a non-genotoxic mechanism [15, 16]. Since the genotoxic potential of BIA 2-093 metabolites, such as BIA 2-194, have not been fully characterized in the *in vitro* mammalian genotoxicity assays due to the use of rat hepatic S9 fraction as a biotransformation system, there are insufficient data to

determine if BIA 2-093 carcinogenic activity can be attributed to a genotoxic or non-genotoxic mechanism of action.

Extensive histopathological examination of the livers of rodents exposed chronically to phenobarbital has revealed that phenobarbital increases the occurrence of eosinophillic foci while not affecting the incidence of the basophilic nodules normally associated with spontaneous tumors [16]. In the Sponsor's carcinogenesis study, there is no dose-related occurrence of eosinophillic foci in the livers of BIA 2-093 exposed mice (Mice with hepatic eosinophillic foci= 2, 4, 1, 2; control, LD, MD, HD, respectively), thereby suggesting that BIA 2-093 may not function in a manner similar to phenobarbital in the induction of hepatic neoplasia. The Sponsor does not address the apparent lack of relationship between BIA 2-093-induced hepatic neoplasia and liver eosinophilic foci.

Supporting the Sponsor's argument, a robust increase in liver weight, suggesting the development of hepatomegaly, is observed in mice exposed chronically to BIA 2-093. In both the 4 week and 13 week mouse studies (093-805, 093-806), liver weight was increased in a dose-dependent manner similar to what occurs in phenobarbital-induced hepatomegaly and neoplasia [16]. It is important to remember that although increased liver weight is a precursor to liver neoplasia in mice exposed to phenobarbital, many of the other characteristics of this type of neoplasia either do not occur in mice exposed to BIA 2-093 or are not satisfactorily demonstrated by the Sponsor to occur.

Overall, the Sponsor has not demonstrated that 1) BIA 2-093 results in induction of CYP450 enzymes via CAR; 2) BIA 2-093 increases the occurrence of eosinophilic foci in the liver and 3) the clastogenic activity of BIA 2-093 is not involved in the generation of hepatic neoplasia in mice. Therefore, the Sponsor's assertion that the carcinogenic potential of BIA 2-093 occurs via a mechanism similar to that of PB carcinogenesis is not supported by the results of the nonclinical studies submitted in NDA 22416.

In summary, although BIA 2-093 is a teratogen, a carcinogen and has multiple toxicological targets, it is the Reviewer's assessment that the overall toxicology profile is not notably different from that of previously-approved antiepileptics. Therefore, it is the Reviewer's conclusion that there are no nonclinical safety signals that would preclude the approval of Stedesa® for the adjunctive treatment of partial-onset seizures in adults with epilepsy.

11. References

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12. Appendix:

Executive CAC Minutes:
Executive CAC
Date of Meeting: October 6, 2009

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
William Taylor, Ph.D., DSPTP, Alternate Member
Lois Freed, Ph.D., DNP, Supervisor
Christopher D. Toscano, Ph.D., DABT, DNP, Presenting Reviewer

Author of Draft: Christopher D. Toscano, Ph.D., DABT

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA # 22-416

Drug Name: STEDESA (Eslicarbazepine acetate, BIA 2-093)

Sponsor: Sepracor, Inc.

Background: Eslicarbazepine acetate (BIA 2-093) is a prodrug that is hydrolyzed *in vivo* to the major active metabolite, S-licarbazepine (BIA 2-194), and two minor metabolites, R-licarbazepine (BIA 2-195) and oxcarbazepine (OXC). BIA 2-093 and its metabolites are antagonists of inactivated voltage-gated sodium channels and voltage-gated calcium channels. The proposed use of STEDESA is as an adjunctive treatment of partial-onset seizures in adults with epilepsy.

Rat Carcinogenicity Study:
The requirement for a rat carcinogenicity study was waived by the division on 7/20/2007. This decision was based on marked differences in *in vivo* metabolism data between rat and human; the rat predominantly metabolizes both BIA 2-093 and BIA 2-194 to OXC, which is a minor metabolite in humans.

Mouse Carcinogenicity Study:
Crl:CD-1 (ICR) BR VAF/Plus mice were administered BIA 2-093 at doses of 0, 100, 250, and 600 mg/kg/day by oral gavage in 0.5% hydroxypropylmethyl cellulose for 104 weeks. The high dose (HD) group was administered 250 mg/kg/day BIA 2-093 for the first week of the study and then administered 600 mg/kg/day for weeks 2-104. The dose escalation in the high dose group was performed to minimize the severe clinical signs associated with the initial administration of high doses of BIA 2-093, and was agreed upon by the ExecCAC (8/28/2003). After consulting with DNP on 7/8/2005, the Sponsor euthanized all surviving males one week before the planned terminus of the study due to the small number of surviving control animals

(11 males at the beginning of week 104). All dose groups consisted of >10 animals/group at termination of the study. A majority of the early decedents were euthanized *in extremis* due to the presentation of severe clinical signs during the study.

Mid dose males, high dose males, and high dose females exhibited statistically significant increases in the incidence of hepatic adenomas and hepatocellular carcinomas. Accompanying the hepatocellular neoplasms were dose-dependent increases in the incidence of hepatic centrilobular hypertrophy and chronic hepatitis.

Executive CAC Recommendations and Conclusions:

- The Committee concluded that the 2-year mouse carcinogenicity study was adequate and that the incidences of hepatic adenomas and hepatocellular carcinomas in males at the mid and high dose and in females at the high dose were drug related.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc: \
/Division File, DNP
Freed/Supervisor, DNP
Toscano/Reviewer, DNP
Demczar/RPM, DNP
/ASeifried, OND IO

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22416	ORIG-1	SEPRACOR INC	SEP-0002093 ESLICARBAZEPINE ACETATE

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/s/

CHRISTOPHER D TOSCANO
04/14/2010

LOIS M FREED
04/14/2010
Please see memo for comments.

PHARMACOLOGY/TOXICOLOGY MEMO TO FILE

Application Number:	22416
Submission Number/code:	012
CDER Stamp Date:	10/7/09
PDUFA Date:	1/30/10
Product:	Eslicarbazepine acetate (BIA 2-093)
Proposed Name:	STEDESA
Applicant:	Sepracor, Inc.
Review Division:	Neurology Products
Reviewer:	Christopher D. Toscano, PhD, DABT
Supervisor/Team Leader:	Lois M. Freed, PhD

MEMO: Submission 012 from Sepracor regarding NDA 22416 was received by CDER on 10/7/09. According to the cover letter, this submission, containing Study 093-874, was submitted in response to "Controlled Substance Pharmacokinetic and Pharmacodynamic Question 1b" which can be found in the Filing Communication (dated 6/12/09). This question was communicated to the Sponsor by the Controlled Substances Staff (CSS). Therefore, a consult request should be sent to CSS for review of Study 093-874.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22416	ORIG-1	SEPRACOR INC	SEP-0002093 ESLICARBAZEPINE ACETATE

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/s/

CHRISTOPHER D TOSCANO
10/09/2009

LOIS M FREED
10/09/2009

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 022416 **Applicant: Sepracor, Inc.** **Stamp Date: 3/30/09**

Drug Name: Eslicarbazepine Acetate (SEP-0002093) **NDA/BLA Type: B1** **Proprietary Name: Stedesa**

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		Format is consistent with the eCTD format described in ICH Guidance M2 EWG entitled "Electronic Common Technical Document Specification". The following sections were not found in the submission a) 4.2.1.4- Pharmacodynamic Drug Interactions
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		Pagination and Indexing are satisfactory.
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		Some study reports are scans of originals. Therefore, text searching and text capturing with Adobe Acrobat are unavailable. All reports are legible.
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		Chronic (6 month) pivotal studies were performed in a species (rat) which does not adequately model the metabolic profile of the human. A second rodent species (mouse) was studied in a 104 week carcinogenesis study. All other required studies for the proposed indication and target population have been submitted and, on the surface, appear to be satisfactory.
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		Capsule and oral gavage chronic studies were performed in rat, mouse and dog.
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		Oral dosing was performed in all pivotal non-clinical studies. Oral route (tablets) is the intended route of exposure in humans.
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		This statement can be found in the non-clinical overview on page 31. Additionally a tabulated summary of all GLP and non-GLP studies can be found on pages 26-30 of this same document.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		The Division has communicated with Sponsor on several previous occasions regarding the design of a juvenile dog toxicity study. This study is not included in this NDA. However, the proposed treatment population in this NDA is adults and therefore a non-clinical juvenile study is not required for this population.
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		Overall, the labeling seems appropriate. However, the accuracy of the statements made in the draft label is a matter of review.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		Study reports qualifying the impurity (b) (4) can be found in Section 4.2.3.7.6.
11	Has the applicant addressed any abuse potential issues in the submission?	X		An abuse potential assessment has been submitted in Section 1.11.4 of the NDA.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?	N/A	N/A	Not Applicable

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.

Not applicable

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

No apparent issues at this point in time.

Christopher D. Toscano, PhD, DABT 5/7/09
Reviewing Pharmacologist Date

Lois M. Freed, PhD
Team Leader/Supervisor Date

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/s/

Lois Freed

5/11/2009 07:40:57 AM