CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

NDA 22-253 & 22-254

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology OND IO
NDA: 22-253, 22-254, —— b(4)
Submission date: September 28, 2007
Drug: lacosamide (tablet)
Sponsor: Schwarz Biosciences
Indication: treatment of Epilepsy as adjunctive therapy in patients with partial onset seizures aged 16 years and older

Reviewing Division: Division of Neurology Products

Introductory Comments: Thes—— NDAs have been submitted for the same drug substance and essentially same indication. The —— NDAs differ in the formulation of the drug product. NDA 22-253 is for a tablet, 22-254 is for an injectable formulation ——.). Another NDA —— was submitted to the Division of b(4)
Anesthesia, Analgesia and Rheumatology Products for the management of neuropathic pain associated with diabetic peripheral neuropathy.

The pharm/tox reviewer and supervisor found the nonclinical information submitted for lacosamide to be sufficient to support its use for the proposed indication.

Reproductive and developmental toxicity:
The sponsor has proposed a pregnancy category of C. The reviewer and supervisor agree with this category.

The reproductive and developmental studies did not indicate that lacosamide was teratogenic but some embryofetal and perinatal mortality and growth deficits were observed. In addition, a juvenile animal study in which rats were treated beginning on postnatal day seven demonstrated some neurobehavioral changes, brain weight decrease and delay in sexual maturation in females. I agree that these studies support a pregnancy category of C. I agree with the supervisory memo that additional embryofetal studies are not needed at this time.

Neurotoxicity:
The reviewer recommended that the sponsor should examine the effects of lacosamide on brain development during the prenatal and early postnatal periods using more sensitive techniques for assessing CNS structure and function than were employed in the standard pre- and postnatal development study.

Because of the apparent neurobehavioral effects observed in the juvenile animal study, and the potential serious consequences of neurotoxicity in children, I agree that further assessment can be requested from the sponsor after approval.

Conclusions:
I concur with the Division pharm/tox conclusion that the nonclinical data support approval of this NDA.

I concur with the recommendation for collecting additional developmental neurotoxicity data post approval.

I agree with the labeling changes suggested in the supervisory memo including deletion of section [redacted] b(4)

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/s/

Paul Brown
7/17/2008 03:17:20 PM
PHARMACOLOGIST

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: July 16, 2008

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

Subject: Lacosamide (SPM927) Tablet (NDA 22-253), Injection (NDA 22-254), ——— b(4)
——— submitted September 28, 2007

Schwarz Pharma has submitted — NDAs to the Division of Neurology Products for lacosamide (SPM927):

- NDA 22-253: Lacosamide tablet for "treatment of Epilepsy as adjunctive therapy in patients with partial onset seizures aged 16 years and older".
  - Lacosamide tablet for the "management of neuropathic pain associated with diabetic peripheral neuropathy" is being reviewed by the Division of Anesthesia, Analgesia and Rheumatology Products under NDA
- NDA 22-254: Lacosamide injection for "treatment of Epilepsy as adjunctive therapy in patients with partial onset seizures aged 16 years and older when oral administration is temporarily not feasible".

b(4)

The nonclinical data in support of these NDAs were submitted under NDA 22-253 and were cross-referenced in NDAs 22-254 and — Review of the nonclinical data submitted in support of the oral formulations was shared by DNP and DAARP.

In DNP, J. Edward Fisher, Ph.D. reviewed the following nonclinical studies:

- Pharmacology (i.e., animal efficacy, mechanism of action) relevant to the epilepsy indication.
- Toxicology
  - Sprague-Dawley rat (14-day i.v.)
  - Beagle dog (14-day i.v.)
- Reproductive toxicology
  - Combined oral fertility and developmental toxicity study in Sprague-Dawley rat

    - Oral embryo-fetal development study in female New Zealand White rabbit
    - Oral pre- and post-natal development study in Sprague-Dawley rat
- Juvenile animal
    - 6-week oral toxicity study in juvenile Sprague-Dawley rat
    - 6-week oral dose-range finding study in juvenile Beagle dog
- Genetic toxicology
    - In vitro bacterial reverse mutation (Ames) assay (2 assays)
    - In vitro mammalian cell gene mutation assay
    - In vivo mammalian cell erythrocyte micronucleus assay
    - In vivo UDS assay in male Fischer rat (hepatocytes)

Terry Peters, D.V.M. reviewed (included in Dr. Fisher's review) the following nonclinical studies:

- Carcinogenicity
    - 2-year oral studies in CD-1 mouse and Sprague-Dawley rat

In DAARP, BeLinda Hayes, Ph.D. reviewed the following nonclinical studies:

- Pharmacology (i.e., animal efficacy, mechanism of action) relevant to the neuropathic pain indication.
- Safety Pharmacology (CNS, cardiovascular, respiratory)
- PK/ADME
- Toxicology
    - Mouse (acute p.o., i.v., 13-week p.o.)
    - Rat (acute p.o., i.v., 13-week p.o., 6-month p.o.)
    - Dog (12-month p.o.)

Based on their review of these data, Drs Fisher and Hayes have concluded that the nonclinical data support approval of lacosamide. However, Dr. Fisher recommends additional studies be conducted post-approval to assess the potential developmental toxicity of lacosamide. Overall nonclinical findings and the basis for the post-approval recommendations will be briefly discussed.

<u>The following is based on information provided in reviews by Drs. Fisher and Hayes. These reviews should be consulted for detailed descriptions and discussion of the nonclinical data.</u>

- Lacosamide ((R)-2-acetamido-N-benzyl-3-methoxypropionamide), a structural analog of D-serine, is a member of a class of functionalized amino acids. The sponsor has proposed that lacosamide exerts anticonvulsant activity via two distinct mechanisms: (1) selective enhancement of "slow inactivation of voltage gated sodium channels without affecting fast inactivation" and (2) "modulation of CRMP-2 activity". CRMP-2 (collapsing response mediator protein-2) is one of four CRMP genes (CRMP 1-4) that have been identified in rat. CRMPs are expressed primarily in the nervous system and are thought to have an important role in development of the CNS. Lacosamide binds to CRMP-2 with a K_D of 5μM. Lacosamide demonstrated no appreciable affinity for a number of other molecular targets (e.g., receptors, transporters).

There does not appear to be clinical evidence that enhancement of slow inactivation, as opposed to fast inactivation, of voltage gated sodium channels conveys any therapeutic benefit. There also does not appear to be documentation that inhibition of CRMP-2 activity has a role in prevention or treatment of epilepsy. A recent publication (Errington AC *et al. Mole Pharm* 73(1):157-169, 2008) funded, at least in part, and co-authored by **the sponsor states that "The molecular mode of action of LCM is still unknown."**

However, there is concern that inhibition of CRMP-2 activity may have adverse effects on neurobehavioral development (discussed further below).

- The **PK/ADME** of lacosamide was assessed in all species used for toxicity testing (i.e., CD-1 mouse, Sprague-Dawley rat, Beagle dog, and New Zealand White rabbit) and in human. Parent was the major drug-related circulating compound in all species. The main circulating metabolite, SPM 12809 (O-desmethyl; no demonstrated pharmacological activity), was also detected in mouse, rat, and dog plasma at levels exceeding those in human plasma (i.e., ≈10-15% of parent compound). Therefore, all animal species tested were acceptable models for assessing the potential for lacosamide-induced toxicity in humans.

- In **safety pharmacology and general toxicology** studies, the primary dose-limiting toxicities in all species (except in the juvenile rat) were CNS-related, and included ataxia, decreased spontaneous motor activity, and convulsions. The only other notable adverse effect was prolongation of the PR and QRS intervals and AV block observed in dog and monkey. (Similar cardiovascular effects have also been reported in human.) Lacosamide exhibited no carcinogenic potential in adequately conducted 2-year studies in mouse and rat.

To support development and approval of lacosamide injection, the sponsor conducted 2-week i.v. toxicity studies in rat and dog. Toxicities were similar to those observed in the oral toxicity studies, i.e., CNS signs were the dose-limiting toxicities. Since, in humans, the i.v. formulation is essentially bioequivalent to the oral tablet except that C_{max} is slightly (≈20%) higher at similar doses, these studies were sufficient to bridge to the oral database.

- No adverse effects were detected in a **combined mating/fertility and embryo-fetal development study in rat** (oral doses up to 200 mg/kg) **or in an embryo-fetal development study in rabbit** (oral doses up to 25 mg/kg) using qd dosing. However, in dose-ranging finding studies, increased resorptions and/or decreases in fetal body weight were observed at the high doses used in the definitive studies and higher (up to 300 and 50 mg/kg in rat and rabbit, respectively). As Dr. Fisher notes, it is unclear why these adverse effects were not observed in the definitive studies. It is possible that the high dose used in each species represents a threshold dose and that the lack of reproducibility is simply due to inter-study variability. Dr. Fisher considered the definitive studies adequate, as conducted. However, **due to the fact that "the maximum plasma drug** exposures tested were relatively low (or uncertain) compared to those expected

clinically", Dr. Fisher recommends that the sponsor be asked to conduct a repeat embryofetal study in one species, if higher plasma exposures can be achieved using b.i.d. dosing.

In the **pre- and post-natal development study in rat**, adverse effects (including prolonged gestation, increases in stillborn pups and postnatal mortality, and decreases in pup body weight) were observed at all oral doses (25-200 mg/kg qd), and prolonged gestation occurred at the high dose. There were no statistically significant effects on postnatal development; however, Dr. Fisher notes that there was "some suggestion of an effect on offspring learning and memory". Adverse effects on neurobehavioral development were also observed in a juvenile rat study, as were decreases in brain weight (absolute and relative). As Dr. Fisher also notes, these effects are consistent with lacosamide's proposed inhibition of CRMP-2, demonstrated in vitro by inhibition of "CRMP-2 mediated effects of neurotrophins...on axonal outgrowth of primary hippocampal cells, at concentrations as low as 1 μM..."

Based on these data, Dr. Fisher has recommended that the sponsor conduct additional studies post-approval to further and more carefully assess the potential for lacosamide to have adverse effects on brain development. Since pediatric use is not being proposed, the most critical periods to assess in rat would be those that correspond to the entire period of human fetal development, i.e., organogenesis through the first 7 days postpartum.

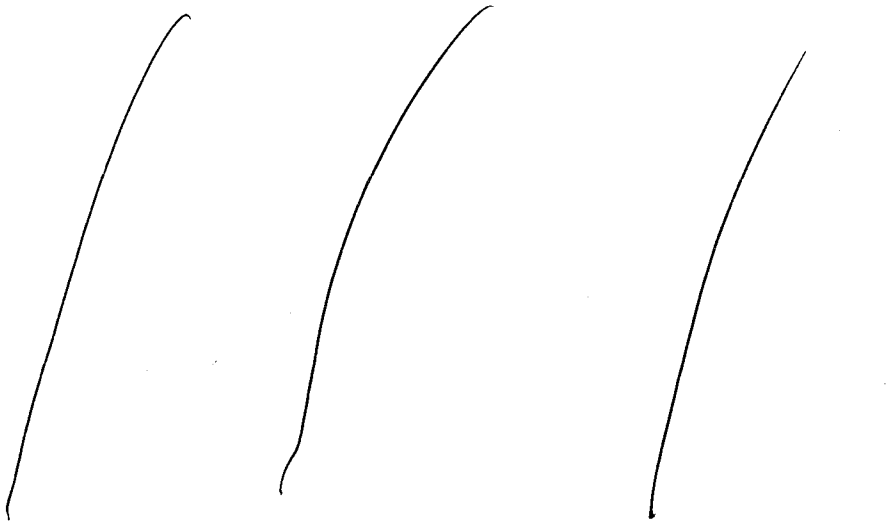
I understand Dr. Fisher's concern regarding the lack of a substantial margin between exposures achieved in rats and those anticipated in humans at the MRHD, and the lack of any plasma exposure coverage in the rabbit. I also agree that the dose-limiting clinical signs observed in both rat and rabbit are probably related to C_{max}, which would suggest that higher plasma exposures could be achieved with b.i.d. dosing. However, an increase in resorptions was also observed in the rat and rabbit dose-range finding studies at doses similar to or ≤ 2 fold higher than those tested in the pivotal studies. This would suggest that substantially higher doses may not be achievable, particularly since there is no basis for assuming that the increases in resorptions are related to C_{max}. In addition, since clearance appears to be saturated at higher doses (e.g., 200 mg/kg, the high dose in the pivotal study) in rat, it is possible that higher systemic exposure resulted from initiation of dosing prior to mating (as in the combined mating/fertility and embryo-fetal study) than could be achieved if dosing were initiated on GD6-7, as is routine in embryo-fetal development studies. For these reasons, it is my opinion that it is unlikely that a better evaluation could be achieved in repeat embryo-fetal studies.

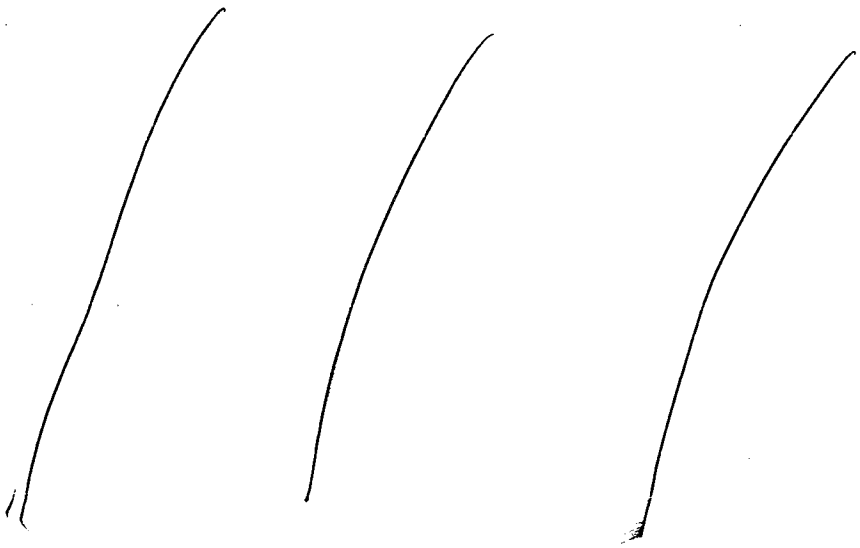
I do concur with Dr. Fisher's recommendation that further assessment of lacosamide's effect on brain development is needed, and that this assessment may be conducted post-approval. Such an assessment should certainly involve dosing in rat throughout the critical periods that correspond to the entire period of human fetal development with, perhaps, direct dosing of the neonate, and, as Dr. Fisher notes, the use of sensitive methods for assessing neurobehavioral function and expanded histopathological examination of the brain. In addition, twice daily dosing should be considered since it more closely mimics the human dosing regimen.

Recommendations

It is my opinion that the nonclinical data support approval of NDAs 22-253, 22-254, [redacted] with a post marketing commitment to conduct an additional assessment of **lacosamide's effect on brain development** (as discussed above). b(4)

Labeling recommendations are as follows:





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_____ Trade Secret / Confidential (b4)

_____ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

ATTACHMENT 1

Plasma exposures (C_{max}, AUC) achieved at the high doses (except as noted) used in the pivotal toxicology studies are provided in the following table (in M-F; data in [brackets] are from dose-range finding, not definitive, studies):

SPECIES	STUDY	SAMPLE TIME	HD (mg/kg)	C_{max} (µg/mL)	AUC (µg*hr/mL)
S-D rat	6-month	D1	90 (MD)	24-25	262-311*
		D1	180	32-30	400-480*
		D182		47-62	488-570*
Beagle dog	12-month	W52	20/25	24-19	142-124*
S-D rat	d-ranging embryo-fetal	D11	300	54	195#
	fertility/embryo-fetal	D11	200	[33]	[115]#
NZW rabbit	d-ranging embryo-fetal	D13	50	47	454!
	embryo-fetal	D13	25	[25]	[153]!
S-D rat	pre/postnatal	D11	200	[33]	[115]#
S-D rat	juvenile	D1	30 (LD)	16-17	113-266*
		D42		11-13	69-123*
		D1	180	140	965-606##
		D42		42	268-290##
Beagle dog	juvenile d-ranging	D1	25	23	90##
		D42		20-24	78-101##
		W103		78-62	230-233*
S-D rat	2-year	W52	160/180	58-60	512-687*
		W104	160/200	50-52	605-737*
CD-1 mouse	2-year	W52	180	80-45	306-193*
		W103		78-62	230-233*
human**	#SP640	steady state	200 mg bid (p.o.)	≈11	≈200
			200 mg bid (i.v.)	≈14	≈200

*0-24 hr, !0-∞, #0-4 hr, ##0-8 hr, **data estimated from AUC(0-12hr) ss data; i.v. data estimated based on 15-min infusion, with C_{max} being ≈20% higher and AUC similar to the same dose of the oral tablet.

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/s/

Lois Freed
7/16/2008 11:00:50 AM
PHARMACOLOGIST

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22-253, 22-254 b(4)
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	9/28/07
PRODUCT:	Lacosamide (SPM927) Tablet, Injection, b(4)
INTENDED CLINICAL POPULATION:	epilepsy
SPONSOR:	Schwarz Biosciences
REVIEW DIVISION:	Division of Neurology Products (HFD-120)
PHARM/TOX REVIEWER:	Ed Fisher
PHARM/TOX SUPERVISOR:	Lois Freed
DIVISION DIRECTOR:	Russell Katz
PROJECT MANAGER:	Jackie Ware

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I. INTRODUCTION AND DRUG HISTORY

NDA number: 22-253 (oral tablet), 22-254 (injection) ——— b(4)

Date of submission: 9/28/07

Sponsor: Schwarz Biosciences

Drug:

Trade name:

Generic name: lacosamide

Code names: ADD 234037; harkoseride; SPM 927

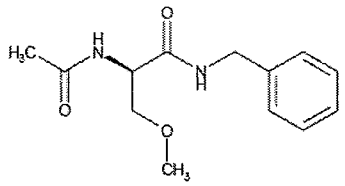
Chemical name: (R)-2-acetamido-N-benzyl-3-methoxypropionamide

CAS registry number: 175481-36-4

Molecular formula: $C_{13}H_{18}N_2O_3$

Molecular weight: 250.3

Structure:



Relevant IND: 57,939

Drug class: sodium channel modulator

Indication: epilepsy

Route of administration: oral (tablets ——— , injection b(4)

Previous reviews: Original IND review dated 3/22/99
CAC-EC reviews dated 7/13/00, 3/1/01, and 6/5/02
CAC-EC minutes dated 8/8/00, 4/24/01, and 7/9/02

Tables and figures are taken directly from the sponsor's submission unless noted otherwise.

II. PHARMACOLOGY

A. BRIEF SUMMARY

Lacosamide (LCM) is a member of a series of functionalized amino acids that were specifically synthesized as anticonvulsant drug candidates. In standard in vitro radioligand binding assays, LCM showed no significant affinity for any of the typical binding sites, including a variety of neurotransmitter, neuropeptide, and growth factor receptors, ion channels, transporters, and intracellular signaling enzymes. However, weak displacement of binding (25% at 10 uM) was observed for the sodium channel site 2. Electrophysiological studies indicated that LCM selectively enhances the slow inactivation of sodium channels without affecting fast inactivation. This was shown to be in contrast to other sodium channel modulators such as lamotrigine, phenytoin, and carbamazepine which enhance fast inactivation. No significant modulation of voltage-gated potassium (KCNQ2/3) or calcium channels (L-, N-, P- and T-type) was detected.

In studies using proteomic affinity-labeling techniques, collapsin-response mediator protein 2 (CRMP-2; also called DRP-2, dihydropyrimidinase-related protein) was subsequently identified as a potential binding target of LCM. In radioligand binding experiments using a cloned human analogue of CRMP-2 expressed in Xenopus oocytes, LCM exhibited a binding affinity of 5 μM.

Due its structural relationship to the endogenous amino acid D-serine, which acts as an NMDA receptor antagonist, LCM was assessed for binding at glutamate receptors. In an initial experiment, 50% displacement of a glycine site antagonist was observed with an IC50 of 5.2 µmol/L. However, in a follow-up study using more specific ligands, LCM (10 µmol/L) did not produce significant (>50%) displacement of specific binding at AMPA, kainate, NMDA (agonist, glycine and phencyclidine binding sites) or glycine receptors isolated from rat brain. But in a functional experiment using recombinant NMDA receptor subtypes, LCM inhibited NMDA- and glycine-induced currents at NR1/2B receptors, albeit with an IC50 of 1.89 mmol/L.

LCM showed anticonvulsant activity in various rodent seizure models, ie, maximal electroshock seizures (MES), 6 Hz seizures, hippocampal kindling, audiogenic seizures (AGS), and self sustaining status epilepticus (SSSE). Lacosamide was inactive against clonic convulsions induced by sc pentylenetetrazol (PTZ), bicuculline, and picrotoxin, but it did inhibit NMDA-induced convulsions in mice. Although inactive against sc PTZ-induced threshold clonic convulsions, LCM elevated the seizure threshold somewhat in the iv PTZ test at the MES ED50. The O-desmethyl metabolite, SPM 12809, and the S-entantiomer of LCM, SPM 6953, were inactive in the MES test at relevant doses.

In vitro investigations of the cardiovascular effects of LCM showed that LCM reduced the action potential duration in cardiac tissue and inhibited sodium current in isolated cells. Effects on sodium current were dependent on membrane potential, with higher inhibition at more depolarized potentials. In vivo studies showed that LCM decreased cardiac conduction. In anesthetized instrumented dogs, LCM induced hypotensive effects characterized mainly by reduced contractility, as indicated by decreases in systolic left ventricular pressure and left ventricular pressure over time (dP/dt) and reduced cardiac output. These effects were accompanied by increases in PR interval and QRS complex duration and by AV block. Similar EEG effects were seen in monkeys, ie, QRS prolongation and AV and ventricular block.

B. MECHANISM OF ACTION

LCM (10–100 μM) showed no significant affinity (>50% inhibition) for any of the typical receptors, channels, or enzymes screened, but did bind weakly (25–50% inhibition) to the sodium channel at the batrachotoxin site 2. LCM did not modulate the uptake of NE, DA, or 5HT into synaptosomes, and did not bind to GABA transporters or influence the activity of GABA transaminases. The

major desmethyl metabolite (SPM 12809) also showed no significant binding to the receptors tested.

In early mechanistic studies, sustained repetitive firing (SRF) of current clamped rat cortical neurons evoked by applying current pulses (750 ms, every 12–14 s) was weakly (78 vs 96% in control) but significantly reduced in frequency by LCM (100 uM) without apparent changes in individual spike properties. This effect was different from that produced by the known sodium channel-blocker phenytoin (100 uM), which produced a large (28 vs 96%) attenuation of spiking during the evoked period of SRF and progressively reduced the amplitude and eventually terminated the AP, but the results suggested that LCM could be acting in part via inhibition of voltage gated sodium channels (VGSCs). When SRF duration was prolonged (10 s) LCM produced significant (EC50: 48 µM) inhibition, but not within the first second of the burst (EC50: 640 µM).

In a study conducted in patch-clamped mouse neuroblastoma cells, which was designed to recruit only fast inactivation (without significant development of slowly inactivated conformations) of the VGSC, significant hyperpolarizing shifts in the fast inactivation voltage curves were produced by the classical anticonvulsants LTG, CBZ, and DPH (all 100 uM). In contrast, LCM (100 µM) did not produce a hyperpolarizing shift in the V50 for inactivation of sodium currents in these cells, ie, the voltage for half maximal inactivation after equilibration with LCM was not significantly different from V50 in control solutions. However, LCM (100 µM) did produce a hyperpolarizing shift in the voltage dependence of slow sodium channel inactivation and promoted channel entry into the slow inactivated state, but did not alter the rate of recovery. The effect of the other drugs on slow inactivation was not tested. (**Table IIB.1**; adapted from Beyreuther et al, CNS Drug Reviews, 13:21-42,2007).

Table IIB.1. Effect of anticonvulsants on the voltage dependence of Na+ channel inactivation

	Control	Lacosamide	Lamotrigine	Carbamazepine	Phenytoin
Fast inactivation V50 [mV]	−66	−65	−72*	−80*	−77*
Slow inactivation V50 [mV]	−43	−58*	n.t.	n.t.	n.t.

n.t., not tested. V50, half maximal reduction of channel availability.
All drugs were applied at a concentration of 100 µM. Steady-state fast inactivation was tested with conditioning prepulses of 500 msec between −120 and −20 mV. For steady-state slow inactivation conditioning, prepulse duration was 5 sec, followed by a 1-sec hyperpolarizing pulse to −100 mV prior to test pulse to −10mV. *$P < 0.05$ vs control.

In another experiment that indicated a selective effect of LCM on slow inactivation, neuroblastoma cells were maintained at a holding potential of -60 mV and depolarized by a 10 ms test pulse to 0 mV at 0.5 Hz. The protocol was then repeated in each cell with a 500 ms hyperpolarizing pulse to -100 mV applied prior to the depolarizing test pulse in order to remove fast inactivation. In all cells tested all four of the anticonvulsants (LCM, LTG, CMZ, DPH; all 100 µM) produced a reduction in Na+ current when the holding potential was -60 mV. For LTG, CBZ, and DPH application of the hyperpolarizing prepulse to -100 mV markedly reduced the blocking action on the channel. In contrast, the inhibition produced by LCM was not significantly altered by the hyperpolarizing prepulse. (**Table IIB.2**; adapted from Beyreuther et al, CNS Drug Reviews, 13:21-42,2007).

Table IIB.2. Inhibition of Na+ current with and without removal of fast inactivation

	Lacosamide	Lamotrigine	Carbamazepine	Phenytoin
% Inhibition of Na+ current with fast inactivation	32	50	71	48
% Inhibition of Na+ current after removal of fast inactivation	29	12*	6*	1*

Availability of Na+ channels was determined in neuroblastoma cells by a 10-msec test pulse to 0 mV from a holding potential of −60 mV. Fast inactivation was removed by a hyperpolarizing pulse to -100 mV (500 msec) prior to the 10-msec test pulse. Concentration of drug was 100 μM for all compounds. *$P < 0.05$ versus % inhibition with fast inactivation.

In affinity-labeling studies, LCM was modified chemically in order to allow covalent crosslinking to potential targets. A set of four LCM-related affinity reagents applied to rat brain fractions led to enrichment of an overlapping set of proteins, including a dihydropyrimidinase-related protein and some additional proteins related to the vesicle release machinery. Although the results suggested that dihydropyrimidinase-related protein may be a target of LCM, they were considered inconclusive, since this type of chemical crosslinking experiment is susceptible to nonspecific side reactions.

Based on the results of this study, a follow-up study was performed in which the human analogue of DRP-2 (CRMP-2) was cloned, expressed in Xenopus oocytes, and the binding of radiolabeled SPM 927 was examined. The results indicated a membrane-associated binding site in oocytes transfected with DRP-2; competitive and specific binding could be observed with a K_D value of about 5 uM. It was pointed out, however, that the expression of DRP-2 could not be monitored directly in this system due to the lack of a functional assay. In cultured rat hippocampal neurons, NT3 and BDNF-stimulated axon growth was inhibited by 1, 10, 100 and 200μM LCM. The positive reference compound wortmannin had a similar inhibitory effect on both neurotrophin-induced effects. These results were thought to provide evidence that LCM may exert some of its pharmacological action via inhibition of CRMP-2.

When LCM (10uM) was evaluated for displacement of various radioligands from glutamate subtype and glycine binding sites (glutamate, AMPA; glutamate, kainate; glutamate, NMDA, agonist; glutamate, NMDA, glycine; glutamate, NMDA, phencyclidine; glutamate, non- selective; and glycine, strychnine-sensitive), no significant responses (≥50% stimulation or inhibition) were seen. In a functional experiment using recombinant NR1/2A and NR1/2B NMDA receptor subtypes expressed in Xenopus oocytes, LCM inhibited NMDA- and glycine-induced currents at NR1/2B but not at NR1/2A receptors with an IC50 of 1.89 mmol/L. Binding was observed to be independent of glycine concentration, indicating noncompetitive antagonism.

C. ANIMAL MODELS OF EPILEPSY

In initial screening, LCM blocked sound-induced seizures in mice with an ED50 of 0.63 mg/kg, ip and protected mice (ED50 = 4.5 mg/kg, ip) and rats (ED50 = 3.9 mg/kg, po) against maximal electroshock (MES)-induced tonic-extension seizures, indicating that LCM is effective in preventing seizure spread (**Table IIC.1**; from Stöhr et al, Epilepsy Res 74:147-54,2007). LCM showed efficacy in the 6-Hz psychomotor seizure test, which is considered a model for treatment-resistant seizures, with an ED50 of 9.99 mg/kg ip. In this model, LCM exhibited additive to synergistic effects with a variety of AEDs (pronounced synergism observed with levetiracetam and CBZ). LCM did not block GTCSs induced by the GABAA-receptor antagonist bicuculline or the chloride channel blocker picrotoxin. LCM was also ineffective against clonic seizures induced by sc bolus injection of pentylenetetrazole (PTZ) in rats and mice. However, LCM significantly increased the threshold for minimal seizures induced by timed iv infusion of PTZ in mice. In the rat hippocampal kindling model, which is thought to predict activity against complex partial

seizures, the ED50 of LCM for reduction of the seizure score from 5 to ≤3 in fully kindled rats was 13.5 mg/kg. LCM (≥10 mg/kg ip) also significantly inhibited kindling development in this model. LCM was also active in a model of status epilepticus, blocking limbic seizures induced by self sustaining status epilepticus (SSSE) in rats. LCM also increased survival and was neuroprotective in this model.

Table IIC.1

Table 1 Summary of anti-convulsant profile of lacosamide in initial screening and differentiation tests in mice and rats

Species and route of lacosamide	Test[a]	Time of test (h)	ED_{50} (mg/kg)	TD_{50} (mg/kg)	95% CI (mg/kg)	Protective index (TD_{50}/ED_{50})[b]
Mice, i.p.	MES	0.5	4.46	–	3.72–5.46	6.0
	Frings AGS	0.5	0.63	–	0.37–0.99	43.0
	6 Hz	0.5	9.99	–	7.73–12.78	2.7
	sc PTZ	0.25	>25	–		n.a.
	sc BIC	1	>50	–		n.a.
	sc PIC	1	>30	–		n.a.
	Rotorod	0.25	–	26.8	25.50–28.00	–
Rats, i.p.	Kindling	0.25	13.5	–	9.11–17.8	n.a.
Rats, p.o.	MES	0.5	3.90	–	2.58–6.20	>128
	sc PTZ	0.5	>250	–	–	
	Minimal motor impairment	0.25–24	–	>500	–	–

[a] At least eight animals were used per treatment group in each test.
[b] PI calculated with TD_{50} obtained in CF#1 mice and ED_{50} in Frings mice.

D. SAFETY PHARMACOLOGY

In Purkinje fibers isolated from male Beagle dog hearts, LCM (1.5, 5, 15, 50, 150 µmol/L) induced concentration-dependent decreases (SS) in action potential duration at 50%, 70% and 90% of repolarization (APD50, APD70, APD90, respectively), both at normal (1 Hz) and low (0.2 Hz) stimulation frequencies (**Table IID.1**). At the high concentration, decreases in APD50, APD70 and APD90 ranged from 30 to 47% at normal frequencies and from 32 to 54% at low stimulation frequencies, respectively. This shortening in action potential duration was associated with reductions in the maximal rate of depolarization (Vmax). At 150 µmol/L, Vmax was reduced by 32% and 36% at 1 Hz and 0.2 Hz, respectively.

Table IID.1 Effect of lacosamide on cardiac action potential in isolated Purkinje fibers
Change in % versus pretreatment values

Stimulation frequency 1 Hz

Lacosamide concentration [µmol/L]	V_{max}	APD_{50}	APD_{70}	APD_{90}
1.5	-6	-1	-2	0
5	-16	-3	-3	-2
15	-6	-9	-8	-5*
50	-9	-24	-19**	-15**
150	-30	-47	-37**	-29**

Stimulation frequency 0.2 Hz

Lacosamide concentration [µmol/L]	V_{max}	APD_{50}	APD_{70}	APD_{90}
1.5	-21	0	-1	-1
5	-21	-2	-1	-2
15	-13	-6	-6	-7
50	-21	-31**	-23**	-20**
150	-33	-52**	-41**	-31**

* = $p \leq 0.05$, ** = $p \leq 0.01$ compared to the vehicle group

In human embryonic kidney cells (HEK293) stably expressing the cardiac SCN5A Na+ channel (= hHNa), LCM (10, 100, 200, 500, 1000 µM) produced a concentration-dependent inhibition of sodium currents (IC50 of 293 µmol/L). The block was incomplete, plateauing at about 70%. When the use-dependence of the block was tested by applying a train of test pulses (10 ms, to -15 mV) from a holding potential of -80 mV until a steady state was reached, the additional block was 1.5% at 0.3 Hz (n=2) and 26% at 3 Hz (n=2) at a LCM concentration of 200 µmol/L. The reference compound lidocaine produced a complete block at 2 mmol/L (n=2).

In CHO cells transiently expressing the human SCN5A channel, LCM (10, 50, 500, 5000 µmol/L; cells clamped to a holding potential of -100 mV) produced a concentration-dependent inhibition of Na+ current (IC50: 112 µmol/L). The maximum block at 5000 µmol/L was 53% for the first and 67% for the average of the last five pulses (use-dependence). In a washout experiment channels were reactivated 150s after drug removal, demonstrating reversibility of the LCM effect. The reference compound lidocaine elicited almost complete block of sodium current at a concentration of 500 µmol/L. The desmethyl-metabolite SPM 12809 did not inhibit the SCN5A mediated current significantly compared to vehicle under similar condition, but when a less negative holding potential of -80 mV was used, a concentration of 100 umol/L SPM 12809 produced a 20% inhibition of the Na+ current.

In isolated human atrial myocytes obtained from human right atrial appendage tissue which had been removed during cardiac surgery, LCM (0.1, 0.3, 1, 3, 10, 100, 5000 µmol/L) produced only minimal Na+ current inhibition (1.4% at 5000 µmol/L) at a hyperpolarized membrane potential (-140 mV). However at a depolarized holding potential (-70 mV), LCM dose-dependently blocked the Na+ current with an IC50 of 67.5 µmol/L and elicited complete block at 5000 µmol/L. Using the same test system, LCM (10, 100, 5000 µmol/L) produced minimal inhibition of Ca^{2+} currents (9.9%). In ventricular myocytes from female guinea-pigs, LCM (15, 50, 150, 500 µmol/L) also failed to affect the current amplitude or current-voltage relationship of the calcium channel.

In voltage clamped human embryonic kidney (HEK293) cells stably expressing the human-ether-a-go-go-related gene (hERG), LCM (10, 100, 300, 3000 µmol/L) produced only a weak inhibition (7%) at the highest concentration.

In an early anesthetized dog study (Study No. 0247DH15.001), dose-related decreases in arterial blood pressure (SAP, DAP, MAP), LVP, and +dP/dt were observed following iv administration of LCM (consecutive 1-min bolus doses of 2.5, 5, 10, and 15 mg/kg; 30 min between doses) to a single male Beagle. MAP was decreased by up to 10, 15, and 27% and LVP by up to 10, 11 and 21%, respectively. Maximum reductions of +dP/dt were 21, 29 and 38%, respectively. The duration of the effect increased with dose. No effects on HR, CO, LVEDP, or ECG parameters were noted in this study.

Another study (#0247DH15.002) in anesthetized dogs (1 male, 1 female), LCM (consecutive doses of 2.5, 5, 10, and 15 mg/kg by iv infusion over 10 minutes; 25 min between doses), D-R

changes in BP (SAP, DAP, MAP), CO, +dP/dt, and LVP were observed. Maximal reductions were 29 and 59% for MAP, 22 and 81% for CO, 33 and 78% for +dP/dt, and 24 and 45% for LVP at 10 and 15 mg/kg, respectively. At the HD, HR was markedly decreased (up to 76%) in both dogs, and the female dog died after about 10 min.. The marked decreases in MAP, CO, and HR appeared at the same time as AV dissociation was observed in the ECG. The ECG evaluation (by ——— stated that LCM "produces hemodynamic changes, including bradycardia, negative inotropy, and lowered arterial blood pressure that can be expected to decrease coronary arterial blood flow. The latter was probably the cause of the one death that occurred. The single lead II electrocardiograms monitored showed that [LCM] causes depression of P wave amplitude and various degrees of AV block. This single lead was insufficient to allow description of the changes in the atrial complexes when the P waves flattened. It appears that atrial conductivity may have been markedly depressed, although this is an uncertain diagnosis." b(4)

In a follow-up study (#0247DH15.003) in a single male dog, 3 consecutive doses of 15 mg/kg LCM were administered iv over ten minutes (30 min between doses). After the initial dose, there was a 23, 22 and 24% decrease in SAP, DAP and MAP, respectively, with no HR change (**Table IID.2**). The 3rd dose produced a 47%, 54%, and 53% reduction in SAP, DAP, and MAP and HR decreased from 129 to 57 bpm (-53%). The ECG evaluation (by ——— stated that LCM "slows intra-atrial conductivity, causes atrioventricular block and dissociation, abolishes surface lead P waves and may cause minor QT prolongation, although the latter is uncertain. The effect on atrial conductivity is like that seen with hyperkalemia or certain drugs that affect potassium ion channels in some way causing hypekalemia-like ECG changes." Regarding the QT effect: "Throughout there was a gradual increase in QT interval duration and STT complex form changes with some ST segment depression. The latter did not exceed normal limits except at the end. These changes also may occur during anesthesia so that the test article cannot be implicated with certainty." b(4)

Table IID.2

Cardiovascular Evaluation of ADD 234037 in a Dog
0247DH15.003

Treatment	Systolic Arterial Pressure (mm Hg)	Dinstolic Arterial Pressure (mm Hg)	Mean Arterial Pressure (mm Hg)	Heart Rate (bpm)
Baseline Values	107	68	83	110
ADD 234037 15 mg/kg, post 10-min infusion	82	53	63	115
30 min post infusion	102	63	77	111
Baseline Values	99	61	75	112
ADD 234037 15 mg/kg, post 10-min infusion	77	48	58	121
30 min post infusion	94	57	71	117
Baseline Values	81	48	60	120
ADD 234037 15 mg/kg, post 10-min infusion	43	22	28	57
40 min post-infusion	77	35	48	66

In the final study (20000376 P) in Beagle dogs (5/sex), LCM (2, 4, 8, and 12 mg/kg iv over 30 sec; 30 min between doses) produced a D-D hypotensive effect due to a cardiodepressant action as suggested by decreases in systolic LVP, -dP/dt, +dLVP/dt, and CO (**Table IID.3**). Increases in PR and PQ interval and QRS complex duration were seen indicating a slowing in both atrio-ventricular and ventricular conductivity at ≥2 mg/kg in females and ≥8 mg/kg in males. One female dog died immediately after receiving the 12 mg/kg dosage due to a marked drop in arterial blood pressure followed by a cardiac arrest. One male dog displayed disturbances of the

electrocardiogram manifesting as junctional rhythm (loss of P wave) at the 12 mg/kg. One female displayed disturbances of the electrocardiogram manifesting as junctional rhythm and junctional premature contractions at 8 mg/kg. There was no effect on QTc in this study. In male dogs plasma LCM concentrations ranged from — to — µg/mL, — to — µg/mL, and from — to — µg/mL at 4, 8 and 12 mg/kg iv, respectively. In female dogs plasma levels ranged from — — µg/mL, — to — µg/mL — to — µg/mL, and from — to — µg/mL at 2, 4, 8 and 12 mg/kg iv, respectively. b(4)

Table IID.3

Effect of lacosamide on cardiac function in anesthetized dogs

Change in % versus predose value

Parameter	2 mg/kg F only	4 mg/kg M/F	8 mg/kg M/F	12 mg/kg M/F
Mean arterial pressure	-3**	-8**/-7**	-10**/-9**	-13**/-10
Systolic LVP	-4**	-9**/-7**	-11**/-10**	-15**/-9
+dP/dt	-8	-17/-12	-18/-11	-27/-12
-dP/dt	-7	-17/-17**	-15/-25**	-23*/-25
Cardiac output	-5**	-6/-7**	-12**/-11**	-13**/-12
Heart rate (absolute HR predose)	+3* (115)	+6/+6** (119/117)	+7/+7** (123/119)	+6/+7 (126/120)
PR interval	+3	+4/+6*	+8**/+5#	+16/+6#
QRS duration	+4	+11**/+8*	+17**/+9	+21#/+13#
QT interval	-2**	-4/-3**	-3/-4	-3/-3
QTc (Fridericia)	-1	-3/-1	-2/-2	-1/-1

F = female, M = male; # parameters did not return to baseline prior to dosing

* = p≤ 0.05, ** p≤ 0.001

Finally, in a study (0247XH15.004) in cynomolgus monkeys (3 males), LCM (10 min iv infusion of 1, 5, 10, and 15 mg/kg [1 monkey] or 30 mg/kg [2 monkeys]; 20 minutes between doses) decreased MAP by up to 50% at 30 mg/kg (**Table IID.4**). HR was decreased by about 30%. ECG changes in the 2 monkeys receiving 30 mg/kg included prolonged P wave duration and decreased P wave amplitude (total loss of P wave), prolongation and increased amplitude of QRS complex, deviation of ST segment, and first and second degree AV block. The ECG evaluation (by — stated that LCM "can slow intra-atrial conduction, cause atrioventricular block, abolish surface P waves, prolong the QRS markedly, and cause intraventricular block. QT interval prolongation associated with the QRS prolongation was present. The QT interval itself was lengthened somewhat beyond the QRS effect, but it is uncertain whether this is a [LCM] effect because of the presence of anesthesia." b(4)

Table IID.4

Effect of lacosamide on ECG, heart rate and blood pressure and in anesthetized monkeys

Primate number	1x30 mg/kg	2x30 mg/kg	3x30 mg/kg	4x30 mg/kg
1	(1+5+10+15) No effect	(1+5+3x15) No effect	N.D.	N.D.
2	No effect MAP- 14%	QRS amplitude↓ QRS duration ↑ MAP -45%	QRS amplitude↓ QRS duration ↑ MAP -50%	QRS amplitude↓ QRS duration ↑↑ AV block
3	QRS duration ↑ ST deviation loss of Pwave MAP – 14% HR -24%	QRS duration ↑ ST deviation AV block MAP -45% HR -27%	QRS duration ↑ ST deviation ventricular block MAP -50% HR -30%	n.d.

N.D. = not determined; MAP = mean arterial pressure, HR = heart rate

Possible neurotoxic effects of LCM were assessed in two rat studies (6958-103). Since early radioligand binding studies indicated a potential interaction with NMDA receptors, the potential for LCM to produce neuronal vacuolization in rat brains was examined. In a study by , LCM was administered at a single ip dose of 50 mg/kg (10-fold anticonvulsant dose in rats), and brains was processed for the determination of vacuolization (6 h after LCM) or cell death (48 h after LCM). Brain tissue from treated rats did not show any signs of vacuolization or neuronal necrosis, while MK-801 produced the expected neuronal vacuolization in the retrosplenial cortex and cell death in different regions of the brain. These results were confirmed in a second study (6958-103). No neuronal vacuolization or cell death were observed at 4 h or 72 h, respectively, following single ip doses of 10 or 50 mg/kg LCM. MK-801 treatment resulted in the expected neuronal vacuolization and necrosis.

b(4)

III. PHARMACOKINETICS/TOXICOKINETICS

ADME reviewed separately by Belinda Hayes, DAARP

A. BRIEF SUMMARY

PK studies showed that LCM was rapidly and well absorbed after oral administration to mice, rats, rabbits and dogs; oral bioavailability was about 80% in rats and dogs based on absorption of radioactivity from radiolabeled drug. The rate and extent of systemic exposure was approximately linear in rabbits and dogs but less than dose proportionate in rodents. In mice, rats and dogs exposure was similar between sexes. Although the half-life was short (t1/2 ≈1.3 and 1.8 hr reported in rat and dog PK studies, but t1/2 of 5 hr determined in rat carcinogenicity study TK report), accumulation was generally observed with repeated dosing in toxicity studies, presumably due to saturation of clearance at higher doses. In vitro protein binding was very low in mouse, rat, dog, and human (unbound fraction 94, 95, 83 and 94%, respectively). Following single oral administration of [14C]-LCM to mice, rats and dogs, radioactivity was rapidly and extensively distributed throughout the tissues with highest concentrations in the gi tract, liver, and kidneys. In the brain few regional differences in the distribution of radioactivity were apparent. Levels of radioactivity in the brain were of the same order of magnitude as in plasma. There was no evidence that radioactivity was specifically binding to any tissue. No significant melanin binding was observed. In pregnant rats, [14C]-LCM-derived radioactivity crossed the placenta and was excreted into the breast milk (levels similar to plasma). Suckling neonates were exposed to LCM-derived radioactivity with a distribution similar to that of maternal tissues in pregnant rats. When 7-day old juvenile rats were orally administered LCM, up to 5-fold higher mean LCM plasma concentrations were determined than in adult animals. No such effect was observed in a dose-range-finding study in 8-week old juvenile dogs. Following oral administration to adult rats for up to 7 days, there were no notable effects on the concentrations of hepatic microsomal protein and cytochrome P450 or on the activities of CYP1A and CYP2B. The major human O-desmethyl metabolite SPM 12809 was found in mouse, rat and dog plasma in vivo. No major differences in systemic exposure to SPM 12809 were observed between species, doses, or sex, but relative exposure to SPM 12809 in animals was generally higher than that determined in clinical trials. The relative exposure to SPM 12809 in animals ranged from 22 to 51% in terms of Cmax and from 25 to 73% in terms of AUC. In human plasma the relative exposure to SPM 12809 was less than 20% in terms of both Cmax and AUC at steady state. Measurable levels of the desacetyl derivative (SPM 6912), which is a minor human metabolite (found in urine but not plasma) [redacted], were also present in mouse and rabbit plasma. Following oral and intravenous administration to mice, rats and dogs, [14C]-lacosamide-derived radioactivity was rapidly eliminated. Similar excretion patterns were observed in all species including human with the majority of radioactivity recovered in urine. Unchanged lacosamide and its O-desmethyl metabolite were the major components in urine of mouse, rat, dog and humans. LCM (R-enantiomer) is a chiral substance, but no bioconversion to the S-enantiomer was observed in rat plasma and dog urine samples. There were no apparent route-related differences in LCM disposition following iv and oral administration of labeled drug to rats and dogs.

b(4)

B. PLASMA LEVELS

b(4)

LCM plasma levels in the toxicity studies are compared to those in humans at [redacted] 300 bid in **Table IIIB.1**. There is little or no AUC coverage. Note that human $AUC_{0\text{-}12h}$ values are used, while $AUC_{0\text{-}24h}$ is given for animals. Data from clinical trial SP588, in which multiple oral doses of 300 mg bid [redacted] were administered to healthy male subjects, were used for comparison with the nonclinical parameters. Combining the two $AUC_{0\text{-}12h,ss}$ values would give an $AUC_{0\text{-}24h}$ of 245 ug.h/ml. This is similar to the $AUC_{0\text{-}\infty}$ value of 231 ug.h/ml reported in clinical study SP587 after a single dose of 600 mg. If 400 mg/day (200 bid) is

the highest dose approved, then an AUC_{0-24h} of 200 ug.h/ml should be used (2X AUC_{0-12h}, ss of 100.3 ug.h/ml; from clinical trial SP640). The sponsor has argued that Cmax is more relevant than AUC, but while true for acute neurotoxic effects such as convulsions, which were dose limiting, it may not be true for other toxicities. The O-desmethyl metabolite (SPM 12809) represents less than 20% of the parent compound in human plasma after repeated oral administration of LCM. The relative exposure to SPM 12809 in % of the lacosamide exposure was 12% in terms of Cmax, ss and 15% in terms of AUC_{0-12h}, ss when taking the molecular weights into account (n = 57 male and female healthy human subjects, 200 mg lacosamide twice daily (12 hours apart) for 6 days, SP640). SPM 12809 is the only major systemically available human metabolite. It was shown to have only weak pharmacological activity.

Table IIIB.1

Comparative pharmacokinetic data following repeated oral administration of lacosamide to mice, rats, dogs and human volunteers

Species Route	Sex	Dose [mg/kg/day]	Parameter of lacosamide [unit] C_{max} [µg/mL]	T_{max} [h]	AUC_{last} [h·µg/mL]
Mouse	Male	30	20.0	0.5	51.5
Oral		60 (NOAEL)	29.0	0.5	97.0
(solution)		120	42.4	0.5	229
		180	53.8	0.5	315
	Female	30	20.4	0.5	46.2
		60 (NOAEL)	27.6	0.5	131
		120	36.6	0.5	170
		180	45.9	0.5	240
Rat	Male	30	11.2	1	140
Oral		90 (NOAEL)	21.8	4	296
(solution)		180	47.0	0.5	488
	Female	30	12.4	1	131
		90 (NOAEL)	33.7	1	339
		180	62.4	1	570
Dog	Male	5	7.1	0.5	31.4
Oral		10 (NOAEL)	15.5	0.8	71.0
(capsule)		25	23.9	1.3	142
	Female	5	7.4	1.3	24.5
		10 (NOAEL)	13.2	1.3	54.6
		25	18.9	1.0	124
Man	Male	8.6 Day 15 (night)	12.6 ± 2.2	3 (1 - 6)	119 ± 18.3
Oral (capsule)		Day 16 (day)	14.5 ± 1.7	1 (1 - 2)	126 ± 17.4

Data presented are after repeated oral administration at the end of a 3-month mouse (individual data), 6-month rat (medians, n=3), 12-month dog (medians, n=2) and 16-day human trial SP588 (means ± SD, n=12, T_{max} median (range)). In man a body weight of 70 kg was assumed (2 x 300 mg per subject/day).
$AUC_{0-24\,h}$ in the mouse, rat and dog, and $AUC_{0-12\,h}$ in man are given.
Data sources: 4.2.3.2.2, LPT 13123/00, Appendix 10-5, 4.2.3.2.6, LPT 13227/00, Appendix 10-5; 4.2.3.2.12, LPT 13196/00, Appendix 10-5; 5.3.3.1.4, SP588, Section 9.4.

IV. **TOXICOLOGY**

A. REPEAT-DOSE ORAL TOXICITY
Oral toxicity studies reviewed separately by Belinda Hayes, DAARP

B. REPEAT-DOSE IV TOXICITY

1. 14-Day Intravenous Injection Toxicity Study of ADD 234037 in Rats (Study No. 6842-101; conducted by [redacted] Report dated 11/20/98; GLP) b(4)

a. Methods

LCM (Lot No. PEH-A-188 (2)) was administered by iv bolus (via tail vein) to SD rats (10/sex/group) at doses of 0, 12.5, 25, or 50 mg/kg/day for 2 weeks. Clinical observations, body weight and food consumption determinations, ophthalmologic exams, organ weights and gross pathological examinations were performed on all animals. Microscopic examinations were conducted on all gross lesions, on a standard list of tissues/organs in the C and HD groups and from rats that died prior to scheduled sacrifice, and on gross lesion, brain, liver, and kidney from LD and MD rats. (There were no TK determinations.)

Doses were based on the results of the single iv dose study in which doses of 25, 50, and 100 were give as an iv bolus and reduced right, limb weakness, ataxia, flattened posture, limb splay, and labored respiration were observed at the 2 highest doses (but no convulsions). There were no deaths but gross pathology findings of pale kidneys and thinned areas in the stomach were found in these groups. The only PK in rats after iv administration came from a single dose study in which male rats received 0, 1, 3 and 10 mg/kg by iv bolus via a jugular catheter. PK parameters are shown in **Table IVB.1.1** below.

b. Results

i. Mortality, clinical signs, body weight

There were no deaths during the study. Clinical signs consisted of moderate to severe hypoactivity and ataxia, appearing within 30 minutes post dose on each study day, at the HD, with females more affected than males. BW gain was decreased in MD females and in HD animals of both sexes (BW decreased by -6.8% and -6.9% in HD males and females, respectively, at end of study; SS; **Table IVB.1.2**). There was a corresponding decrease in food consumption in these groups.

ii. Clinical Pathology

RBC parameters were increased slightly in HD males and females (probably due to hemoconcentration). Serum Alk Phos, ALT, AST, and creatinine were increased in treated males (**Table IVB.1.3**). The ALT and AST changes primarily reflected the markedly increased activities in one HD male (C02730), but in no case were histological correlates observed. Similar changes were not seen in females. Urinalysis indicated a diuretic effect, with increased (SS) urine volume in HD males (+247%) and females (+402%) and lower concentrations of urinary solutes (potassium, sodium, creatinine and urea nitrogen) and lower specific gravity.

iii. Ophthalmology

There were no findings considered treatment-related.

iv. Pathology

There were no T-R gross pathologic or histopathologic findings.

c. Conclusions

Intravenous administration of 12.5, 25, or 50 mg/kg LCM to SD rats for 2 weeks decreased BW gain, produced clinical signs of acute neurotoxicity (ataxia and hypoactivity), and produced increased RBC parameters, enzyme activities (SGOT, SGPT, ALK Phos), and diuretic effects, primarily at the MD and HD. There were no histopathological changes related to treatment.

Table IVB.1.1

Nominal Dose [mg/kg]	SPM 927 C_{max} [µg/mL]	SPM 927 AUC_{last} [h µg/mL]	SPM 927 t_{half} [h]	SPM 12809 C_{max} [µg/mL]	SPM 12809 t_{max} [min]	SPM 12809 AUC_{last} [h µg/mL]
1	1.717	3.43	1.9	0.111	135	na
3	4.482	12.8	1.9	0.234	135	1.13
10	19.533	40.1	2.2	0.949	135	4.97

na not applicable

Table IVB.1.2

BODY WEIGHT CHANGE MEANS AND STANDARD DEVIATIONS (G)

	SEX:	MALE				FEMALE			
	GROUP:	1	2	3	4	1	2	3	4
	DOSE:	0	12.5	25	50	0	12.5	25	50
AY	UNITS:	MG/KG/DAY	MG/KG/DAY	MG/KG/DAY	MG/KG/DAY	MG/KG/DAY	MG/KG/DAY	MG/KG/DAY	MG/KG/DAY
1	N	10	10	10	10	10	10	10	10
	MEAN	49	49	48	41*	24	19*	17*	19*
	S.D.	2.9	2.4	2.3	4.8	3.9	4.7	4.5	4.6
5	N	10	10	10	10	10	10	10	10
	MEAN	45	47	45	41	23	25	21	17
	S.D.	3.2	3.5	4.8	6.9	9.2	5.2	4.9	3.9
1-15	N	10	10	10	10	10	10	10	10
	MEAN	94	96	93	81*	47	44	39*	36*
	S.D.	3.8	5.3	6.0	11.3	10.0	5.9	6.3	6.1

* SIGNIFICANTLY DIFFERENT FROM CONTROL VALUE, P ≤ 0.05.

Table IVB.1.3

SUMMARY OF CLINICAL CHEMISTRY DATA

14-DAY INTRAVENOUS INJECTION TOXICITY STUDY OF ADD 234037 IN RATS
MALES

GROUP		GLUCOSE - MG/DL	UREA N - MG/DL	CREAT - MG/DL	ALK P - U/L	T CHOL - MG/DL	AST - U/L
		DAY	DAY	DAY	DAY	DAY	DAY
		16	16	16 RT	16	16	16 RT
1	(0 MG/KG/DAY)						
	N	10	10	10	10	10	10
	MEAN	113	17	.5	158	95	93
	S.D.	15.3	2.4	.05	14.1	17.2	10.3
2	(12.5 MG/KG/DAY)						
	N	10	10	10	10	10	10
	MEAN	108	19	.5	158	102	99
	S.D.	17.1	3.0	.07	15.3	21.1	16.9
3	(25.0 MG/KG/DAY)						
	N	10	10	10	10	10	10
	MEAN	110	18	.5	178*	103	94
	S.D.	6.9	1.7	.07	17.7	11.6	6.1
4	(50.0 MG/KG/DAY)						
	N	10	10	10	10	10	10
	MEAN	114	17	.6*	183*	111	141
	S.D.	16.3	1.8	.05	19.5	19.4	145.5

GROUP		ALP - U/L	T PROT - G/DL	ALBUMIN - G/DL	GLOBULIN - G/DL	A/G - RATIO	CALCIUM - MG/DL
		DAY	DAY	DAY	DAY	DAY	DAY
		16 RT	16 RT	16	16	16	16 RT
1	(0 MG/KG/DAY)						
	N	10	10	10	10	10	10
	MEAN	49	6.2	4.6	1.6	2.91	11.2
	S.D.	6.6	.31	.27	.14	.321	.67
2	(12.5 MG/KG/DAY)						
	N	10	10	10	10	10	10
	MEAN	55	6.1	4.5	1.7	2.73	11.3
	S.D.	7.9	.13	.16	.18	.312	.31
3	(25.0 MG/KG/DAY)						
	N	10	10	10	10	10	10
	MEAN	51	6.2	4.6	1.6	2.86	11.4
	S.D.	6.1	.21	.23	.12	.315	.28
4	(50.0 MG/KG/DAY)						
	N	10	10	10	10	10	10
	MEAN	89	6.1	4.6	1.5	3.04	11.4
	S.D.	113.6	.12	.14	.14	.340	.55

RT - DATA ANALYZED FOLLOWING RANK TRANSFORMATION.

2. 14-Day Intravenous Injection Toxicity Study of ADD 234037 in Beagle Dogs (Study No. 98793; conducted by [redacted] Report Dated 5/5/98; GLP) b(4)

a. Methods

LCM (Lot No. PEH-188 (2)) was administered by iv bolus (via cephalic vein) to Beagle dogs (4/sex/group) at doses of 0, 4, 8, or 16 mg/kg/day for 2 weeks. Endpoints included clinical observations, body weight, food consumption, ophthalmology, ECG (including heart rate), clinical pathology variables, toxicokinetics, organ weights, gross pathology, and histopathology (all tissues from C and HD, liver, kidneys, brains, and gross lesions from all groups).

Doses were based on the results of a preliminary rising dose tolerance study in which Beagle dogs were dosed (iv bolus) with 15, 22.5, and 30 mg/kg. There were no deaths during the study. Clinical signs consisted of ataxia, hypoactivity, tremor, emesis, and salivation at ≥15 mg/kg, and convulsions and prostration at ≥22.5 mg/kg. There were no TR findings observed at necropsy.

b. Results

i. Mortality, clinical signs, body weight

There were no deaths during the study. Clinical signs (emesis, tremors and hind limb weakness) were observed in HD males only. One HD male

additionally showed salivation, ataxia, lethargy, seizures, and lack of coordination on several study days. Signs started about 2 minutes postdose and resolved within 2 hours of dosing. Males were more affected than females. There were no BW or food consumption effects.

ii. Ophthalmology

There were no findings considered treatment-related.

iii. Electrocardiography

A single HD female animal (SAN 31) was diagnosed with second degree AV heart block at the Day 13 evaluation, which was not apparent on the ECG taken prior to dosing. All other ECGs were said to be within normal limits.

iv. Clinical Pathology

There were no clear T-R changes in hematological or clinical chemistry parameters. An apparent increase in urine output with concomitant urodilution of creatinine, sodium, potassium, and chloride, and elevated urea nitrogen was noted at the HD in both sexes.

iv. Plasma levels

Plasma concentrations of LCM were measured up to 8 hr post-dose and TK parameters determined (**Table IVB.2.1**). The t1/2 values ranged from 1.6 to 1.9 hours.

vi. Pathology

There were no T-R gross pathologic or histopathologic findings.

c. Conclusions

Intravenous administration of LCM to Beagle dogs at doses of 4, 8, or 16 mg/kg/day for 2 weeks decreased produced clinical signs of acute neurotoxicity (tremors, hind limb weakness, ataxia, lethargy, seizures, incoordination), ECG changes (AV block in 1 female), and diuretic effects at the HD. There were no histopathological changes related to treatment.

Table IVB.2.1 Mean PK parameters in 14-day iv toxicity study in dogs

		Males		Females	
Dose (mg/kg/d)	**Day**	**Cmax (µg/mL)**	**AUC (µg.h/mL)**	**Cmax (µg/mL)**	**AUC (µg.h/mL)**
4	0	6.6	22.2	8.0	22.0
	13	6.8	25.3	6.5	22.3
8	0	11.7	41.0	13.9	53.4
	13	13.0	45.9	15.3	53.7
16	0	24.3	97.2	24.0	90.9
	13	26.1	106.5	25.4	93.9

C. GENETIC TOXICOLOGY

1. Bacterial Reverse Mutation Assay - ADD 234037 (Study No. G97BR23.502, Dated 11/18/97, Conducted by [redacted] GLP) b(4)

In the first Ames test, LCM (Lot No. 234037) was tested in 4 Salmonella typhimurium strains (TA 98, TA 100, TA 1535 and TA 1537) and 1 E coli strain (WP2 uvrA). A plate incorporation test with and without metabolic activation was performed. Five concentrations in the range of 100 to 5000 µg lacosamide per plate were used. Cytotoxicity was detected only with tester strain TA 98 in the absence of S9 activation at 5000 µg/plate in the preliminary cytotoxicity test. No precipitate was observed. LCM had no mutagenic effects in any of the tested strains, with or without metabolic activation (**Table IVC.1.1**). The positive controls produced the expected response.

Table IVC.1.1

Test Article Id : ADD 234037
Study Number : G97BR23.502 Experiment No : B1

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg)	TA98	TA100	TA1535	TA1537	WP2 uvrA
0.0	38 ± 2	134 ± 19	12 ± 3	7 ± 3	16 ± 4
100	27 ± 5	142 ± 10	11 ± 1	8 ± 3	13 ± 1
333	31 ± 7	125 ± 12	9 ± 1	6 ± 1	15 ± 5
1000	25 ± 4	112 ± 10	7 ± 3	7 ± 4	14 ± 4
3333	31 ± 5	140 ± 10	9 ± 1	9 ± 4	13 ± 2
5000	22 ± 2	121 ± 16	6 ± 2	7 ± 3	11 ± 4
Pos	288 ± 17	874 ± 53	566 ± 28	860 ± 171	214 ± 43

Liver Microsomes: Rat liver S9

Dose (µg)	TA98	TA100	TA1535	TA1537	WP2 uvrA
0.0	35 ± 4	145 ± 13	11 ± 2	8 ± 2	17 ± 5
100	29 ± 6	130 ± 10	9 ± 1	8 ± 5	16 ± 1
333	40 ± 3	154 ± 21	13 ± 3	9 ± 4	14 ± 2
1000	35 ± 10	142 ± 8	13 ± 3	13 ± 4	12 ± 1
3333	28 ± 5	147 ± 5	13 ± 3	11 ± 1	16 ± 3
5000	29 ± 4	141 ± 6	10 ± 5	7 ± 4	10 ± 2
Pos	702 ± 52	705 ± 37	85 ± 11	132 ± 12	383 ± 96

0.0 = Vehicle plating aliquot of 50 µL
Pos = Positive Control concentrations as specified in Materials and Methods section.

b(4)

2. Bacterial Reverse Mutation Assay – SPM 927 (Report No. IPL-R 000603, Dated 12/1/00, Conducted by [redacted] . GLP)

In a second independent Ames test, LCM (Batch No. KK 02457) was tested in strains TA 98, TA 100, TA 1535 and TA 1537 and in E coli strains WP2 and WP2 uvrA. Plate incorporation and pre-incubation tests each with and without metabolic activation were performed. Five concentrations in the range of 15 to 1500 µg per plate were used. The maximum concentration was selected based on solubility. No interfering cytotoxicity was detected up to the maximum tested concentration of 1500 µg/plate. No precipitate was observed. LCM showed no mutagenic activity in any of the tested strains, with or without metabolic activation (**Tables IVC.2.1a & b**). The positive controls produced the expected response.

Tables IVC.2.1a

	TA 1535		TA 1537		TA 98		TA 100		WP2(pkM101)		WP2uvrA(pkM101)	
	DOSE µg/plate	mutants /plate	DOSE µg/plate	mutants /plate	DOSE µg/plate	mutants /plate	DOSE µg/plate	mutants /plate	DOSE µg/plate	mutants /plate	DOSE µg/plate	mutants /plate
Postive control	(a)	837.7	(a)	1437	(a)	839.3	(a)	897.3	(a)	241.3	(a)	1139
	0	11.5	0	3.7	0	17.3	0	93.3	0	71.7	0	184
TEST	15	12.3	15	6	15	17	15	99	15	72.7	15	155.3
COMPOUND	50	8	50	3.3	50	10.3	50	98	50	71.3	50	179.3
without	150	5.3	150	4.7	150	14.7	150	81.3	150	72.3	150	178
S9 mix	500	8.3	500	4	500	15	500	83.7	500	68.3	500	184.3
	1500	14.7	1500	4	1500	16.3	1500	82	1500	72.7	1500	200
Postive control	(b)	381.3	(b)	119.3	(b)	1089	(b)	1131	(b)	190	(b)	1433
TEST	0	5.3	0	4	0	11	0	69	0	49	0	188
COMPOUND	15	7	15	5	15	13	15	86	15	70	15	198
with	50	7	50	3.3	50	9	50	82	50	79	50	223
S9 mix	150	6	150	3.3	150	13	150	75	150	79	150	198
without	500	6.7	500	2.3	500	11	500	88	500	89	500	201
preincubation	1500	8.7	1500	3.3	1500	11	1500	78	1500	77	1500	177

Positive reference compounds (µg/plate) :
(a) TA1535 and TA100 : Sodium azide 1 ; TA1537 : 9-amino-acridine 50 ; TA98 : 2-nitrofluorene 2
WP2(pkM101): Mitomycin C 0,125; WP2uvrA(pkM101): potassium dichromate 15
(b) TA 1535, TA1537, TA98, TA100 : 2-anthramine 2 ; TA102: benzo(a)pyrene5

Tables IVC.2.1b

	TA 1535		TA 1537		TA 98		TA 100		WP2(pkM101)		WP2 uvrA(pkM101)	
	DOSE µg/plate	mutants /plate	DOSE µg/plate	mutants /plate	DOSE µg/plate	mutants /plate	DOSE µg/plate	mutants /plate	DOSE µg/plate	mutants /plate	DOSE µg/plate	mutants /plate
Positive control	(a)	644.3	(a)	1068	(a)	707.7	(a)	556.7	(a)	400.7	(a)	990
	0	22.2	0	6.8	0	16	0	109.8	0	71.7	0	163.2
TEST	15	22.7	15	5.7	15	15	15	85.3	15	58.7	15	148.7
COMPOUND	50	24.7	50	4.3	50	14.7	50	110.7	50	62	50	162.3
without	150	28.7	150	3	150	14	150	106	150	67	150	192.3
S9 mix	500	23.7	500	3.7	500	10.7	500	102	500	67.3	500	173.3
	1500	31.7	1500	3.3	1500	16	1500	107.3	1500	61	1500	184
Positive control	(b)	129.3	(b)	328	(b)	1533	(b)	1232	(b)	117	(b)	921
TEST	0	19	0	5.3	0	19	0	95	0	73	0	204
COMPOUND	15	21.3	15	4.3	15	21	15	88	15	83	15	194
with	50	20.7	50	6.3	50	24	50	80	50	88	50	218
S9 mix	150	23.3	150	3.7	150	24	150	79	150	80	150	220
with	500	23.3	500	5.7	500	23	500	73	500	80	500	207
preincubation	1500	24	1500	5.7	1500	22	1500	83	1500	65	1500	195

Positive reference compounds (µg/plate) :
(a) TA1535 and TA100 : Sodium azide 1 ; TA1537 : 9-amino-acridine 50 ; TA98 : 2-nitrofluorene 2
WP2(pkM101): Mitomycin C 0,125; WP2uvrA(pkM101): potassium dichromate 15
(b) TA 1535, TA1537, TA98, TA100 : 2-anthramine 1; WP2(pkM101) and WP2uvrA(pkM101): benzo(a)pyrene 2,5

b(4)

3. In Vitro Mammalian Cell Gene Mutation Test (study # G97BR23.704, conducted by completed 1/8/99, GLP)

LCM (lot #s TED-D-50 and PEH-A-188(2)) was tested in the L5178Y/TK+/- mouse lymphoma assay in the absence and presence of S9. In the preliminary toxicity assay, no visible precipitate and no toxicity (growth <50% of the solvent control (DMSO)) was observed at any concentration (up to 5000 ug/mL with a 4-hour exposure and 2500 ug/mL with a 24-hour exposure) with or without S9 activation.

Concentrations from 500 to 4000 ug/mL were used in the non-activated assay and concentrations from 1000 to 5000 ug/mL were used in the S9- activated assay. In the non-activated assay, a dose-related increase in mutant frequency was seen at ≥2000 µg/mL (**IVC.3.1**). Concentrations >4000 ug/mL were considered excessively toxic. In the S9-activated cultures, a D-R positive response was also observed at ≥2000 µg/mL(**Table IVC.3.2**). Toxicity was observed at doses >2000 ug/mL without activation and at 5000 ug/mL with S9 activation.

In the independent repeat assay with a 24-hour exposure period without activation, at concentrations from 250 to 3000 ug/mL, a positive response was again seen at ≥2000 µg/mL but cell toxicity was considered extreme (13% and 18% growth compared to C) at 3000 ug/mL (**Table IVC.3.3**). Toxicity was observed at doses >2000 ug/mL.

The data on colony size distributions showed an increase in the frequency of small colonies consistent with a clastogenic mechanism (**Figure IVC.3.1**).

Table IVC.3.1

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
TREATED WITH ADD 234037
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

Test Article Concentration (µg/mL)		TFT Colonies Counts			Mean	VC Colonies Counts				Mean	Mutant Freq.[a]	Induced Mutant Freq.[b]	% Total Growth[c]
Solvent	1	24	31	35	30 ±5	132	175	163	157	±18	38		
Solvent	2	31	39	44	38 ±5	132	136	157	142	±11	54		
Mean Solvent Mutant Frequency= 46													
500	A	39	41	34	38 ±3	132	124	131	129	±4	59	13	80
500	B	38	28	39	35 ±5	172	166	162	167	±4	42	-4	108
1000	A	34	50	35	40 ±7	164	119	126	136	±20	58	12	74
1000	B	28	33	42	34 ±6	156	124	119	133	±16	52	6	83
2000	A	63	57	60	60 ±2	120	135	126	127	±6	94	49	45
2000	B	48	64	63	58 ±7	106	106	107	106	±0	110	64	35
3000	A	84	80	74	79 ±4	129	117	111	119	±7	133	87	30
3000	B	76	68	107	84 ±17	109	144	131	128	±14	131	85	36
4000	A				++	65	71	83	73	±7			5
4000	B				++	71	85	100	85	±12			7
Positive Control - Methyl Methanesulfonate (µg/mL)													
10		148	135	123	135 ±10	84	109	84	92	±12	293	247	41
20		126	125	143	131 ±8	46	45	32	41	±6	641	595	12

Solvent = DMSO

A and B or 1 and 2 are duplicate cultures

++ - Too toxic to count, total growth <10%

[a] - Mutant frequency (per 10^6 surviving cells)=(Average # TFT colonies / average # VC colonies) x 200

[b] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[c] - % total growth = (% suspension growth x % cloning growth) / 100

APPEARS THIS WAY
ON ORIGINAL

Table IVC.3.2

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS TREATED WITH ADD 234037 IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

Test Article Concentration (µg/mL)		TFT Colonies Counts	Mean	VC Colonies Counts	Mean	Mutant Freq.[a]	Induced Mutant Freq.[b]	% Total Growth[c]
Solvent	1	30 51 33	38 ±9	151 148 155	151 ±3	50		
Solvent	2	46 57 44	49 ±6	148 139 116	134 ±13	73		
Mean Solvent Mutant Frequency= 62								
1000	A	70 63 60	64 ±4	157 133 145	145 ±10	89	27	105
1000	B	55 79 87	74 ±14	168 137 160	155 ±13	95	33	109
2000	A	92 90 97	93 ±3	135 126 129	130 ±4	143	81	81
2000	B	89 104 126	106 ±15	146 133 93	124 ±23	172	110	83
3000	A	111 111 130	117 ±9	120 107 133	120 ±11	196	134	66
3000	B	112 118 131	120 ±8	122 144 140	135 ±10	178	116	75
4000	A	130 133 110	124 ±10	151 114 136	134 ±15	186	124	65
4000	B	125 149 165	146 ±16	144 106 112	121 ±17	243	181	60
5000	A	106 99 91	99 ±6	106 104 118	109 ±6	180	119	41
5000	B	143 148 143	145 ±2	125 125 129	126 ±2	229	167	50
Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/mL)								
2.5		156 178 166	167 ±9	117 123 109	116 ±6	287	225	70
4		160 188 173	174 ±11	107 100 100	102 ±3	339	278	44

Solvent = DMSO

A and B or 1 and 2 are duplicate cultures

[a] - Mutant frequency (per 10^6 surviving cells)=(Average # TFT colonies / average # VC colonies) x 200

[b] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[c] - % total growth = (% suspension growth x % cloning growth) / 100

APPEARS THIS WAY ON ORIGINAL

Table IVC.3.3

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
TREATED WITH ADD 234037
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
Independent Repeat Assay (24-hour Exposure)

Test Article Concentration (µg/mL)		TFT Colonies Counts			Mean	VC Colonies Counts				Mean	Mutant Freq.[a]	Induced Mutant Freq.[b]	% Total Growth[c]
Solvent	1	28	35	31	31 ±3	175	171	169	172	±2	37		
Solvent	2	35	22	41	33 ±8	165	171	181	172	±7	38		
Mean Solvent Mutant Frequency= 37													
250	A	29	37	21	29 ±7	184	162	133	160	±21	36	-1	93
250	B	34	42	38	38 ±3	182	145	175	167	±16	45	8	97
500	A	35	28	37	33 ±4	177	184	151	171	±14	39	2	105
500	B	30	20	27	26 ±4	164	179	148	164	±13	31	-6	100
1000	A	32	31	17	27 ±7	171	153	165	163	±7	33	-4	86
1000	B	21	20	13	18 ±4	159	168	172	166	±5	22	-16	82
2000	A	40	28	39	36 ±5	178	184	132	165	±23	43	6	56
2000	B	52	33	42	42 ±8	144	145	135	141	±4	60	23	44
3000	A	64	55	71	63 ±7	116	103	116	112	±6	113	76	13
3000	B	64	58	53	58 ±4	137	103	109	116	±15	100	63	18
Positive Control - Methyl Methanesulfonate (µg/mL)													
2.5		118	117	98	111 ±9	138	159	138	145	±10	153	116	79
5		133	143	148	141 ±6	122	97	91	103	±13	274	236	45

Solvent = DMSO

A and B or 1 and 2 are duplicate cultures

[a] - Mutant frequency (per 10^6 surviving cells)=(Average # TFT colonies / average # VC colonies) x 200

[b] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[c] - % total growth = (% suspension growth x % cloning growth) / 100

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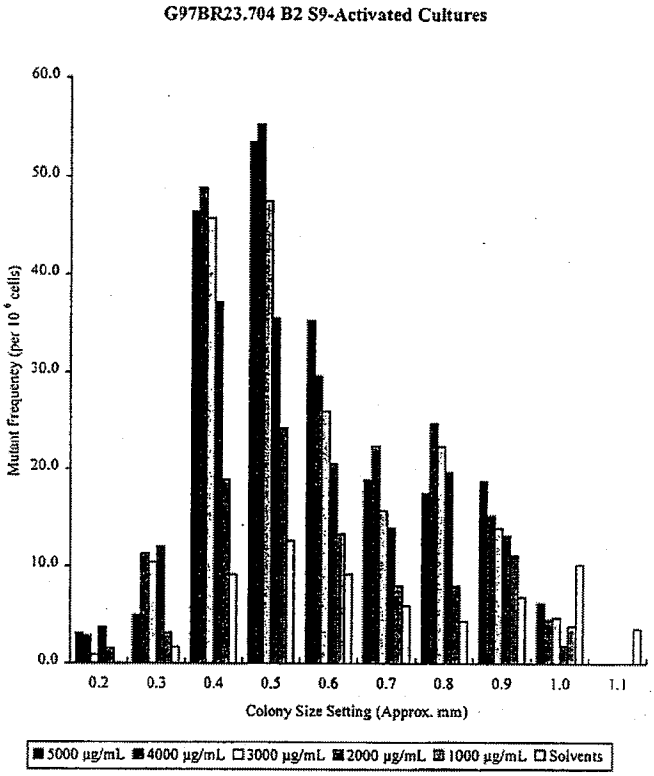
ON ORIGINAL

Figure IVC.3.1

Colony Size Distribution in the Presence of Metabolic Activation

(Test Article-Treated Cultures Compared with Solvent Control)



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b(4)

4. Mammalian Erythrocyte Micronucleus Test (Study No.: G97BR23.123, conducted by , completed 1/16/98, GLP)

ICR mice (5/sex/dose) were administered ip doses of 0 (vehicle: 0.5% carboxymethylcellulose), 50, 100 or 200 mg/kg lacosamide (lot # PEH-A- 188 (2) and bone marrow was collected at 24 hours (all groups) and 48 hours (control and 200 mg/kg groups) post-dose. Dose selection was based on a preliminary toxicity study in which mortality was seen at doses >200 mg/kg (**Table IVC.4.1**). In the definitive study, 1 female out of 10 HD mice died. Clinical signs at all doses included convulsions, prostration, irregular breathing, lethargy and lack of coordinated movements. The ratio of PCEs to total erythrocytes was decreased by 5 to 12% (NS) in some treated groups compared to the C. Lacosamide produced no clear increase in the number of micronucleated PCEs in either sex (**Table IVC.4.2**), although there were some small increases, particularly after 48 hr. The positive control produced the expected effect. TK measurements in a separate study in mice were 63, 125, and 221 ug/ml for Cmax, and 108, 264, and 725 ug.h/ml for AUC at doses of 50, 100, and 200 mg/kg, respectively (mean male and female combined; no sex difference).

Table IVC.4.1

Clinical Signs Following Dose Administration of ADD 234037
Toxicity Study

G97BR23.123

Treatment	Clinical Observation	Number of Animals Affected/Total Number of Animals Dosed		Number of Animals Died/Total Number of Animals Dosed	
		Males	Females	Males	Females
ADD 234037, 50 mg/kg	Convulsions Prostration Irregular breathing Lack of coordinated movements	5/5 5/5 5/5 5/5	5/5 5/5 5/5 5/5	0/5	0/5
ADD 234037, 100 mg/kg	Convulsions Prostration Irregular breathing Lack of coordinated movements Lethargy	5/5 5/5 5/5 5/5 5/5	5/5 5/5 5/5 5/5 5/5	0/5	0/5
ADD 234037, 200 mg/kg	Convulsions Prostration Irregular breathing Lack of coordinated movements Lethargy	5/5 5/5 5/5 5/5 4/5	5/5 5/5 5/5 5/5 2/5	0/5	0/5
ADD 234037, 400 mg/kg	Convulsions	5/5	5/5	5/5	5/5

Table IVC.4.2

Summary of Bone Marrow Micronucleus Study Using ADD 234037

Treatment	Sex	Time (hr)	Number of Mice	PCE/Total Erythrocytes (Mean +/- sd)	Change From Control (%)	Micronucleated Polychromatic Erythrocytes Number per 1000 PCEs (Mean +/- sd)	Erythrocytes Number per PCEs Scored[1]
0.5% Carboxymethylcellulose							
20 mL/kg	M	24	5	0.52 ± 0.08	---	0.4 ± 0.65	4 / 10000
	F	24	5	0.53 ± 0.04	---	0.4 ± 0.22	4 / 10000
ADD 234037							
50 mg/kg	M	24	5	0.46 ± 0.14	-12	0.4 ± 0.42	4 / 10000
	F	24	5	0.56 ± 0.05	6	0.7 ± 0.57	7 / 10000
100 mg/kg	M	24	5	0.53 ± 0.03	2	0.4 ± 0.42	4 / 10000
	F	24	5	0.55 ± 0.06	4	0.6 ± 0.42	6 / 10000
200 mg/kg	M	24	5	0.46 ± 0.04	-12	0.3 ± 0.45	3 / 10000
	F	24	5	0.56 ± 0.04	6	0.2 ± 0.27	2 / 10000
CP,							
60 mg/kg	M	24	5	0.49 ± 0.03	-6	21.7 ±10.95	*217 / 10000
	F	24	5	0.54 ± 0.02	2	14.0 ± 4.65	*140 / 10000
0.5% Carboxymethylcellulose							
20 mL/kg	M	48	5	0.65 ± 0.04	---	0.6 ± 0.65	6 / 10000
	F	48	5	0.59 ± 0.10	---	0.4 ± 0.55	4 / 10000
ADD 234037							
200 mg/kg	M	48	5	0.62 ± 0.03	-5	0.9 ± 0.42	9 / 10000
	F	48	5	0.64 ± 0.06	8	1.2 ± 0.91	12 / 10000

[1]*, p≤0.05 (Kastenbaum-Bowman Tables)

5. Measurement Of Unscheduled DNA Synthesis (UDS) in Rat Hepatocytes using an In Vivo Procedure With SPM 927 (Report # IPL-R 000801, conducted by [redacted], dated 12/15/00, GLP)

b(4)

In an effort to assess the relevance of the positive results seen in the in vitro mouse lymphoma assay, an in vivo DNA repair assay was conducted. In a preliminary toxicity assay, groups of 4 male Fischer rats were given single oral doses of 125, 200, 320, or 500 mg/kg. Clinical signs consisted of decreased spontaneous motor activity, loss of righting reflex, clonic convulsions (1/4 at 500 mg/kg), and ptosis. One HD rats was found dead. Based on the severity of signs at 320 mg/kg, 200 mg/kg was selected as the HD. A lower dose of 100 mg/kg was also tested.

For the UDS test, 3 males/dose and expression time were given oral doses of 100 and 200 mg/kg LCM (batch # KK 02457). Solvent and positive control groups were included. Results are shown in **Table IVC.5.1**. Over the two experiments, net nuclear grain count (NNG) values at the two doses were less than zero, ie, below the threshold for a positive response. However, percentages of cells in repair were increased somewhat (NS, non-D-R) compared to the respective controls. For cells in repair, group mean net nuclear grain count (NNG>5) values were also increased somewhat (NS) compared to solvent controls. Based on the established criteria, it was concluded in the study report that LCM did not induce a proliferative effect in rat livers under the experimental conditions. Peak plasma concentrations of LCM were 20.6, 27.0 and 45.8 ug/ml at 1 hour post-dose after 50, 100, 200 mg/kg, respectively.

Table IVC.5.1

In Vivo UNSCHEDULED DNA SYNTHESIS TEST IN RAT HEPATOCYTES

RECAPITULATIVE TABLE

Animal species: Fischer rats
Sex: male
Route: Oral
Number of animals per group: 3

ASSAY I: 12-16 HOUR EXPRESSION TIME

DOSE	Net nuclear grain count NNG		% cells in repair NNG≥5		Net nuclear grain count of cells in repair NNG≥5		% cells in S-phase
	Mean	± sd	Mean	± sd	Mean	± sd	Mean
NEGATIVE CONTROL 0.5% carboxymethyl cellulose	-2.81	4.96	4.16	2.86	6.81	0.81	0.3
LOW DOSE 100 mg/kg	-2.86	4.70	3.44	2.96	6.65	1.06	0
HIGH DOSE 200 mg/kg	-2.18	4.58	5.34	3.14	7.38	2.05	0.1
POSITIVE CONTROL 2-acetamidofluorene 25 mg/kg	9.04	5.78	75.38	12.05	11.36	4.42	0

ASSAY II: 2-4 HOUR EXPRESSION TIME

DOSE	Net nuclear grain count NNG		% cells in repair NNG≥5		Net nuclear grain count of cells in repair NNG≥5		% cells in S-phase
	Mean	± sd	Mean	± sd	Mean	± sd	Mean
NEGATIVE CONTROL 0.5% carboxymethyl cellulose	-3.32	4.72	2.30	2.43	6.46	1.00	0.4
LOW DOSE 100 mg/kg	-3.43	4.66	2.87	2.50	7.37	0.97	1.3
HIGH DOSE 200 mg/kg	-4.07	5.06	2.42	2.26	10.27	3.87	0.6
POSITIVE CONTROL Dimethylhydrazine 10 mg/kg	13.03	8.09	83.44	3.88	15.34	6.88	0

D. CARCINOGENICITY (Reviewed by Terry Peters, DNP)

1. Study title: 104-Week Carcinogenicity Study of SPM 927 by Oral Administration to CD-1 Mice

Key study findings: Clinical signs demonstrated that higher doses would not have been tolerated. No significant clinical signs were appreciated at 20 mg/kg/d of SPM 927. At 60 mg/kg/d, ataxia and reduced activity were reported for the first 8 weeks on study but abated until Week 35 when decreased activity was noted in males and Week 54 when it was noted in females. Neither tremors nor convulsions were reported for this dose group. In the animals treated with 180 mg/kg/d, ataxia, decreased activity and "abdominal position" were observed for almost all of the animals throughout the study. Tremors were reported during the first 11 weeks on study as well as clonic convulsions in all treated animals at this dose. These tremors/convulsions started 5-20 minutes after dosing and lasted for up to 3 hrs. While the males in this group showed a -10% difference in body weight change when compared to controls, it appears that the severe clinical signs did not appreciably affect the physiology of the affected animals.

No additional adverse effects were recorded for any of the animals on study to include hematology, clinical chemistries, organ weights, gross or histologic pathology.

Adequacy of the carcinogenicity study and appropriateness of the test model: The CD-1 mouse is considered an appropriate model for evaluation of carcinogenic potential. The high dose (180 mg/kg/d) in this study elicited significant clinical signs of toxicity (tremors, clonic convulsions, ataxia, hypoactivity) so this dose is considered the maximum tolerated dose. No increase in mortality was found at any dose tested.

Evaluation of tumor findings: There were no increases in tumor incidence or type in any dose group.

Study no.: 13124/00
Volume and page #:. Electronic submission
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 7/24/01
GLP compliance: German GLP regulations
QA report: Yes
Drug, lot #, and % purity: WE11837 ("corresponds to SIFA batch no. 537.1008 as well as 537f01005") at 99.6% purity
CAC concurrence: yes (4/24/01; see review and minutes)

Methods
Doses: 0 (tap water), 0 (vehicle), 20, 60 or 180 mg/kg/d
Basis of dose selection: The thirteen week dose range-finding study with doses of 0, 30, 60, 120 and 180 mg/kg/d. The NOEL was 30 mg/kg/d (per sponsor) as 60 mg/kg/d elicited ataxia, hypoactivity and "abdominal position" for the first 2 weeks on study. At the higher doses, these signs were noted on most study days. No effects on hematology, clinical chemistries, or histopathology were determined. In the study, the Cmax comparison at 120 mg/kg/d with the human Cmax at 300 mg/dose bid provided a ratio of approximately 3 (per sponsor). b(4)
Species/strain: CD-1/ [redacted].CD®-1 (ICR)BR mice
Number/sex/group (main study): 50
Route, formulation, volume: Oral gavage in aqueous 0.5% hydroxypropyl-methylcellulose gel at 10 mL/kg
Frequency of dosing: Daily

Satellite groups used for toxicokinetics or special groups: 15/sex/group treated for 52 weeks after which they were euthanized but not examined further.
Age: 6 weeks at study initiation
Animal housing: Individually
Restriction paradigm for dietary restriction studies: N/A
Drug stability/homogeneity: Fresh mixtures were prepared daily and were determined to be correct and homogeneous.
Dual controls employed: One tap water control, one vehicle control
Interim sacrifices: Satellite animals
Deviations from original study protocol: The body weight range of the animals at study initiation was slightly ≥10% of the mean weights. This did not have an effect on study outcome.

Observation times
Mortality: Twice/day
Clinical signs: From 7 am to 3:45 pm on weekdays and less often on weekends. They were examined immediately pre- and post-dosing. Animals were palpated weekly from Week 27 for masses.
Body weights: Weekly up to Week 13 and every 2 weeks thereafter
Food consumption: As for body weights
Hematology: At Week 52 and 104 from the first 10 animals/sex/group. A differential count was performed on samples from controls, high dose and premature decedent animals if euthanized in extremis.
Clinical chemistries: As for hematology from the second 10 animals/sex/group
Ophthalmoscopy and auditory evaluations: Weeks 26, 52 and 104 from the first 10 animals/sex/group prior to the daily dose. Auditory acuity was checked for these animals at the same time.
Organ weights: Adrenals, brain, heart, kidney, liver, lungs, ovaries, prostate, spleen, testes, thymus, uterus. "Organ weights which differed considerably from the normal values due to detected or likely tumors or other defined pathological changes were parenthesized and excluded from the statistics."
Histopathology: A complete tissue list was examined for each animal on study. Additionally, the brain sections were elaborated to contain frontal cortex and basal ganglia, parietal cortex and thalamus and cerebellum and pons.
Bone marrow myeloid: erythroid ratios were determined for animals from Groups 1, 2, and 5 only at terminal sacrifice.
Peer review: Yes of 10% of animals from all groups and at least 10% of all tumors. No target tissues were peer reviewed "as no target organs were identified in this study".
Toxicokinetics: Blood samples were taken from 3/sex/dose from the satellite animals in Week 26, from satellite and selected (Groups 3, 4, and 5) main study animals during Week 52 (0.5, 1, 3, 8 and 24 hrs post-dosing) and all main study animals at necropsy. The samples were analyzed by LC-MS/MS methodology by [redacted] (first 2 time points) and [redacted] ,inal samples). b(4)

Results

Mortality: Survival for the 20 mg/kg females was significantly higher than for all other groups. The reason for this difference is not evident. Survival among the other groups was essentially equal.

Mean Survival Times (Test Weeks) for Mice Treated for Up to 104 Weeks

Group	Premature Decedents Males	Females	All Animals Males	Females
1	83.3	73.5	91.6	87.3
2	84.6	83.4	94.0	92.0
3	81.0	78.6	92.0	96.5
4	83.9	81.8	91.1	93.4
5	77.2	71.7	89.4	89.0

Survival Rates at Study Termination

Group	Survival rates (%) Males	Females
1	38	44
2	46	40
3	46	68
4	34	50
5	44	52

Clinical signs: No significant clinical signs were appreciated at 20 mg/kg/d.

At 60 mg/kg/d, ataxia and reduced activity were reported for the first 8 weeks on study. These signs abated until Week 35 when decreased activity was noted in males and Week 54 when it was noted in females. However, some decreased activity was also noted in controls. However, the data did not show any evidence of tremors.

In the animals treated with 180 mg/kg/d, ataxia, decreased activity and "abdominal position" were observed for almost all of the animals throughout the study. Tremors were reported during the first 11 weeks on study as well as clonic convulsions in all treated animals at this dose. These tremors/convulsions started 5-20 minutes after dosing and lasted for up to 3 hrs.

Body weights: No significant effects on body weights were found except in the high dose males, where mean body weight was reduced by 10.6% at the end of the study. Statistical significance was reached in this group ($p \leq 0.01$ when compared to vehicle controls) at most time points. All other groups had ≤2.9% difference in body weight compared to control at week 103.

Total Body Weight Gain for Mice Treated with SPM927

Parameter	Vehicle M	F	20 mg/kg/d M	F	60 mg/kg/d M	F	180 mg/kg/d M	F
Mean BW (gms) Week 1	30.6	23.0	30.8	23.0	30.9	23.4	30.4	23.5
Mean BW Week 103	40.7	32.5	39.5	31.9	39.5	31.6	36.4	31.9
Difference in gms	10.1	9.5	8.7	8.9	8.6	8.2	6.0	8.4
Difference in %	33.0	41.3	28.2	38.7	27.8	35.0	19.7	35.7
Total % difference	--	--	-13.9	-6.3	-14.9	-13.7	-40.6	-11.6

Food consumption: No treatment-associated intergroup differences were discussed.

Ophthalmoscopy and auditory evaluations: No treatment-related differences were conveyed.

Hematology and clinical chemistries: No significant intergroup differences were engendered. Bone marrow smears were evaluated as described above and no significant inter-group differences were found.

Gross pathology: No treatment-related lesions were seen at gross necropsy.

Histopathology:

Non-neoplastic: The sponsor reported no treatment-related lesions in any dose group.

Neoplastic: No increased tumor incidences were recorded for any dose group. The major tumor type in males and females was pleomorphic lymphoma but it was distributed throughout the dose groups so no treatment effect is assigned.

Summary Tumor Incidence

	Males					Females				
Group #	1	2	3	4	5	1	2	3	4	5
Total #/group	50	50	50	50	50	50	50	50	50	50
Total 1° tumors	33	39	36	25	21	31	24	26	27	33
Total # of animals with tumors	23	29	25	23	18*	26	29	23	24	25
Animals with multiple tumors	9	9	10	2	3	5	5	3	2	7
Benign	12	22	19	16	15	10	14	10	5	14
Malignant	21	17	17	9	6	21	20	16	22	19
Malign with mets.	5	4	9	4	1	19	16	11	13	17

* Significantly different from controls ($p \leq 0.05$)

Toxicokinetics: Cmax increased in a less than dose-proportional fashion. No accumulation was found and no gender differences were described. A small amount of test article was found in 2/12 control samples but at different time points. This finding is not considered toxicologically significant.

Mean PK Parameters for Mice Treated Up to 104 Weeks

		Males			Females		
Dose (mg/kg/d)	**Week**	**Cmax (µg/mL)**	**Tmax (Hr)**	**AUC last (µg.h/mL)**	**Cmax (µg/mL)**	**Tmax (Hr)**	**AUC last (µg.h/mL)**
20	26	12.6	0.5	36.3	11.8	0.5	27.8
	52	16.4	0.5	47.8	16.4	0.5	40.2
	103	12.6	0.5	28.9	10.5	0.5	33.5
60	26	29.9	0.5	103	29.0	1	86.3
	52	33.9	0.5	103	37.0	0.5	95.3
	103	29.8	0.5	99.0	30.4	0.5	92.9
180	26	54.4	0.5	269	44.7	0.5	225.0
	52	78.8	0.5	306	44.7	1	193.0
	103	78.5	0.5	230	62.5	0.5	233.0

2. Study title: 104-Week Carcinogenicity Study of SPM 927 by Oral Administration to CD® Rats

Key study findings: Clinical signs demonstrated that higher doses would not have been tolerated. No significant clinical signs were appreciated at 40 or 80 mg/kg/d of SPM 927. At the high dose (Males: 160 mg/kg/d; females: 160/180/200 mg/kg/d), clonic convulsions with/without "abdominal position" were reported in Weeks 4-18 in about 1/3- 1/2 of the animals; hypoactivity was noted from Weeks 19-29 in this dose group. Once the dose in females was increased to 180 mg/kg/d, an increase in "abdominal position" was recorded for a couple of days in approximately1/2 of the animals. Similarly, when the dose was again increased to 200 mg/kg/d, increased "abdominal position" and hypoactivity were reported for most of the females and persisted for approximately 2 weeks. These signs were elicited 5-20 minutes post-dosing and persisted for up to 2 hrs. At 60 mg/kg/d, ataxia and reduced activity were reported for the first 8 weeks on study but abated until Week 35 when decreased activity was noted in males and Week 54 when it was noted in females. Neither tremors nor convulsions were reported for this dose group. In the animals treated with 180 mg/kg/d, ataxia, decreased activity and "abdominal position" were observed for almost all of the animals throughout the study. Tremors were reported during the first 11 weeks on study as well as clonic convulsions in all treated animals at this dose. These tremors/convulsions started 5-20 minutes after dosing and lasted for up to 3 hrs. While the males in this group showed an -8% difference in body weight when compared to controls at the end of the study, it appears that the severe clinical signs did not appreciably affect the physiology of the affected animals.

No additional adverse effects were recorded for any of the animals on study to include hematology, clinical chemistries, organ weights, gross or histologic pathology. No neoplastic lesions were found related to treatment with SPM 927.

Adequacy of the carcinogenicity study and appropriateness of the test model: CD rats are an acceptable model to determine the carcinogenic potential of pharmaceuticals. At the high dose, significant clinical signs of toxicity were reported (clonic convulsions, "abdominal position" and hypoactivity) so the top dose is considered a maximal tolerated dose even though no increase in mortality was observed.

Evaluation of tumor findings:

Study no.: 13295/00
Volume and page #: Electronic submission
Conducting laboratory and location: b(4)

Date of study initiation: 8/6/01
GLP compliance: German and US GLP compliance
QA report: Yes
Drug, lot #, and % purity: WE11837 (corresponds to SIFA batch #537.1008 and 537f01005 at 99.6% purity
CAC concurrence: Yes (8/8/00; see review and minutes)

Methods

Doses: 0 (tap water), 0 (vehicle- 0.5% aqueous hydroxypropyl-methylcellulose gel), 40, 80 and 160 mg/kg/d (males) or 160/180/200 mg/kg/d (females). The doses in females were increased in Week 51 and on Day 516 due to lack of sufficient toxicity. These increases were done with CAC concurrence.

Basis of dose selection: The NOAEL for the 4 and 13 week toxicity studies was set at 100 mg/kg/d as 300 mg/kg/d resulted in increased mortality.

Species/strain: CD® rats

Number/sex/group (main study): 50

Route, formulation, volume: Oral gavage at 5 mL/kg in 0.5% hydroxypropyl-methylcellulose gel

Frequency of dosing: Daily

Satellite groups used for toxicokinetics or special groups: TK:10/sex/dose for treated groups to be euthanized at 52 weeks without further evaluation

Age: 6 weeks at study initiation

Animal housing: Individually

Restriction paradigm for dietary restriction studies: N/A

Drug stability/homogeneity: Acceptable

Dual controls employed: Yes

Interim sacrifices: Satellite animals only without examination

Deviations from original study protocol: None significant

Observation times

Mortality: Twice daily

Clinical signs: "Regularly" throughout the working day. Beginning in Week 27, the animals were palpated weekly for masses.

Body weights: Weekly for the first 13 weeks on study and every 2 weeks thereafter

Food consumption: As for body weights

Ophthalmic and auditory evaluations:

Hematology and clinical chemistries: In Weeks 52 and 104 from 10 animals/sex/dose; bone marrow myeloid: erythroid ratios were determined for the control and high dose groups.

Urinalyses: Weeks 52 and 104 from 10 animals/sex/group. Samples were taken from 5 males from each dose group on Day 561 for urinary metabolite determination.

Gross pathology: All animals on study

Organ weights: Adrenals, brain, epididymides, heart, kidney, liver, lungs, lymph nodes (cervical, mesenteric), ovaries, pituitary, prostate, spleen, testes, thymus, thyroid/parathyroid, uterus.

Histopathology: All tissues from all animals were evaluated microscopically.

Peer review: yes

Toxicokinetics: Samples were taken in Week 26 from satellite animals, Weeks 52 and 59 from satellite and "selected" main study animals and in Week 104 from all remaining animals on study. Analysis was by LC-MS/MS methodology.

Results

Mortality: No significant inter-group differences were found with respect to mortality.

Survival Rates at Study Termination in Rats Treated for Up to 104 Weeks

Group	Survival rates (%) Males	Females
1	58	60
2	64	68
3	68	70
4	74	58
5	58	54

Clinical signs: No systemic toxicity was evidenced by clinical signs in the low or mid dose groups.

At the high dose (Males: 160 mg/kg/d; females: 160/180/200 mg/kg/d), clonic convulsions with/without "abdominal position" were reported in Weeks 4-18 in about 1/3- 1/2 of the animals;

hypoactivity was noted from Weeks 19-29 in this dose group. Once the dose in females was increased to 180 mg/kg/d, an increase in "abdominal position" was recorded for a couple of days in approximately 1/2 of the animals. Similarly, when the dose was again increased to 200 mg/kg/d, increased "abdominal position" and hypoactivity were reported for most of the females and persisted for approximately 2 weeks. These signs were elicited 5-20 minutes post-dosing and persisted for up to 2 hrs.

Body weights: No significant body weight effects were reported for the low or mid dose animals or high dose females on study. The high dose males showed a slight difference from controls that reached statistical significance ($p \leq 0.01$) towards the end of the study (mean BW 7.9% below vehicle control at week 103). The mean body weight change was decreased by an average of 3.8% in this group (max: -8.7 % when compared to controls). This difference is not considered toxicologically significant.

Total body weight gain (TW 0 to TW 103[18])

Parameter	Group 2 Vehicle control		Group 3 40 mg/kg		Group 4 80 mg/kg		Group 5 160 mg/kg	Group 5 160/180/200 mg/kg
	Males	Females	Males	Females	Males	Females	Males	Females
Mean b.w. [g] in TW 0	203.6	149.7	206.2	150.4	208.0	149.8	208.2	147.4
Mean b.w. [g] in TW 103	580.3	357.2	565.0	385.5	571.1	370.8	534.3	348.1
Difference [g] TW 103 – TW 0	376.7	207.5	358.8	235.1	363.1	221.0	326.1	200.7
Difference [%] TW 103 - TW 0	185.0	138.6	174.0	156.3	174.6	147.5	156.6	136.2
Total difference to vehicle control [%] in TW 103 (based on g values)	-	-	-4.8	13.3	-3.6	6.5	-13.4	-3.3

Food consumption: No significant intergroup differences were observed.

Ophthalmic and auditory evaluations: No treatment-associated differences from controls were discussed.

Hematology and clinical chemistries: No intergroup differences in hematologic parameters were perceived.

The mid and high dose males and females showed significant differences from controls in ALT values up to Week 39. While the differences reached statistical significance, they did not reach biologic significance as the maximal increase was +86% in the Week 13 samples for females. All other increases were ≤49% so are not considered toxicologically significant. Slightly increased liver weights were also determined but no histologic correlates were described.

Urinalyses: No meaningful differences between groups were found.

Gross pathology: No treatment-attributable gross lesions were described.

Organ weights: Increased absolute and relative liver weights were found at doses ≥80 mg/kg/d but the differences were ≤20% when compared to control values. This difference is not considered biologically significant, especially as no histologic correlates were noted.

Histopathology:

Non-neoplastic: No increases in lesions attributable to treatment were found in any dose group.

Neoplastic: There were no increases in tumors reported for any group. The majority of the tumors were pituitary adenomas (males and females), Leydig cell tumors in males and mammary gland fibromas in females. Tumor incidence for these tumors decreased with increasing doses of SPM 927.

Summary Tumor Incidence

	Males					Females				
Group #	1	2	3	4	5	1	2	3	4	5
Total #/group	50	50	50	50	50	50	50	50	50	50
Total 1° tumors	59	61	66	63	54	86	98	94	73	66
Total # of animals with tumors	37	35	39	38	32	39	44	40	42	34*
Animals with multiple tumors	12	16	17	18	14	25	34	30	23*	17**
Benign	45	49	53	50	41	67	72	74	58	52
Malignant	14	12	13	13	13	19	26	20	15	14
Malign with mets.	8	6	5	1	7	7	11	9	5	3

* Significantly different from controls ($p \leq 0.05$)
** Significantly different from controls ($p \leq 0.01$)

Toxicokinetics: Cmax and AUC increased in a less than dose-proportional fashion and no accumulation was evidenced over the duration of the study. No significant gender differences were appreciated, even though the doses in females were increased twice during the study.

Mean PK Parameters for Rats Treated Up to 104 Weeks

		Males			Females		
Dose (mg/kg/d)	**Week**	**Cmax (µg/mL)**	**Tmax (Hr)**	**AUC last (µg.h/mL)**	**Cmax (µg/mL)**	**Tmax (Hr)**	**AUC last (µg.h/mL)**
40	26	17.6	0.5	169	27.5	0.5	185
	52	20.8	0.5	177	22.2	0.5	190
	104	15.2	0.5	180	17.9	0.5	202
80	26	27.6	0.5	299	34.4	1	429
	52	34.8	1	333	46.6	1	371
	104	27.1	0.5	373	29.3	0.5	342
160	26	48.9	2	514	57.0	0.5	659
160(M)180(F)	52	57.9	2	512	59.6	0.5	687
160(M)200(F)	104	49.5	1	605	51.6	0.5	737

E. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

1. Combined Oral (Gavage) Fertility and Developmental Toxicity Study of Harkoseride (ADD 234037) in Rats (Protocol No. 1108-003; report dated 12/6/00; conducted by —— b(4)

a. Methods

Rats (Sprague-Dawley; 25/sex/grp) received 0 (vehicle: 0.5% methylcellulose), 25, 70, or 200 mg/kg LCM orally (by gavage) either beginning 28 days before cohabitation (maximum 21 days) and continuing through the day before sacrifice (males) or beginning 15 days before cohabitation and continuing through gestation day (GD) 17 (females). (There were no apparent effects on reproductive organs in the general toxicology studies.) Clinical observations, body weights and food consumption were recorded during the dosing and postdosing periods. After cohabitation, males were sacrificed, and a gross necropsy was performed. Male reproductive organs (testis, epididymis, seminal vesicles, and prostate) were weighed, and cauda epididymal sperm concentration and motility were evaluated (CASA). On GD 20, females were sacrificed, C-sectioned, and a gross necropsy was performed. The number of corpora lutea was recorded and the uterus was examined for pregnancy, implantation sites, live and dead fetuses, and early and late resorptions. Each fetus was weighed and examined for sex and gross external alterations; 1/2 of fetuses were examined for visceral abnormalities (Staples method) and 1/2 were examined for skeletal alterations after staining (alizarin red S).

Strain: — CDBR VAF/Plus b(4)
Drug lot: KK02457

Dose selection:
Doses were based on an embryofetal range-finding study in which pregnant rats (8/grp) were given 0, 100, 200, or 300 mg/kg on GD 7-17 (based on doses in subchronic rat toxicity study). There were no deaths. Treatment-related clinical signs included decreased motor activity, ataxia, impaired or lost righting reflex, urine-stained abdominal fur, and excess salivation at the 2 highest doses (occurred throughout dosing period). BW gain was significantly reduced throughout the dosing period at the MD and HD (20 and 60% compared to C over GD 7-20). The number of early resorptions, number of dams with any resorptions, and percent resorbed conceptuses per litter were increased in the MD and HD (1.0, 0.7, 1.6, 2.2 % resorbed/litter in C, LD, MD, and HD). Fetal body weights were reduced at the HD (30% compared to C). No fetal gross alterations were reported (skeletal and visceral not evaluated). TK data from this study is shown in **IVE.1.1** below. PK values for the LD and HD were comparable to those measured in the 13 week rat toxicity study (AUCs reanalyzed using the same time interval, ie, 0-4 hrs). On Day 1 in the 13 week study, Cmax values were about 22 and 40 ug/ml, and AUC_{0-8} values were about 150 and 200 ug.h/ml at 100 and 300 mg/kg, respectively. Plasma levels were still high at the last sampling time (8 hr) in the 13-week study, so the AUCs were underestimated.

b. Results

i. Mortality and Clinical Observations

Three male deaths were attributed to intubation accidents (1 C on day 55, and 2 HD on days 6 and 55). All other animals survived to scheduled sacrifice.

Dose dependent (D-D) increases in observations of impaired righting reflex and excess salivation occurred in MD and HD males and females. Additional T-R observations in HD animals were increased incidences of ataxia, decreased

motor activity, impaired proprioceptive positioning and limited use of the hindlimbs, splayed hindlimbs, urine-stained abdominal fur (females), tiptoe walk, and lost righting reflex.

D-D increases in incidences of impaired righting reflex and excess salivation persisted in MD and HD females during gestation, and ataxia and urine-stained abdominal fur, as well as one or two observations of tip-toe walk or impaired proprioceptive positioning were seen at the HD during gestation.

ii. Body Weight

Body weight (BW) gains for the premating (Day 1-28) and total treatment periods were significantly decreased in HD males (27 and 14% compared to C, respectively). However, these differences reflect a transient effect that was mostly seen during the first week of treatment (Day 1-8; SS) and then diminished during the rest of the treatment period (**Figures IVE.1.1**; **Table IVE.1.2**). Terminal BW was slightly but SS decreased in HD males (4% compared to C).

In females, BW gain was significantly decreased at the HD during the premating period (89% compared to C over Days 1 to 15), resulting in decreased BW on Day 15 of dosing (5% below C). BW gain was transiently decreased in HD females on GDs 0 to 7, but BW gains were comparable for the overall gestational treatment period, and BWs were not different on GD 20 (**Figure IVE.1.2**; **Tables IVE.1.3-4**).

iii. Male and female reproductive indices

Estrous cycling, mating and fertility parameters were unaffected treatment (**Tables IVE.1.5-6**). All rats in cohabitation mated. There were no effects on male reproductive organ weights or on caudal epididymal sperm motility, count, and density.

iv. Litter parameters

No treatment effects on C-sectioning or litter parameters were apparent (**Table IVE.1.7**). No increases in fetal abnormalities (external, visceral, and skeletal malformations or variations) were seen (**Table IVE.1.8**).

v. Necropsy

There were no effects on testes weights or other necropsy observations.

3. Conclusions

Treatment of male and female rats with LCM (oral doses of 20, 75, or 200 mg/kg) prior to and during mating and through organogenesis (GD 17) resulted in clinical signs of toxicity and transient reductions in parental body weight gain at the HD but produced no apparent adverse effects on mating and fertility or on C-sectioning and litter parameters. (The increased resorption seen at 200 mg/kg in the dose range-finding study was not replicated.) Although maternal toxicity was less evident during the gestational treatment period in females, presumably due to tolerance development during the premating period, the dose range-finding study indicated that dose selection was appropriate for the gestational period and TK data indicate no change or increases in plasma drug levels over time; therefore, the study can be considered adequate under once daily dosing conditions.

Table IVE.1.1 Pharmacokinetic parameters in rat embryofetal dose range-finding study

		Pharmacokinetic Parameter											
		Tmax (hr)			Cmax (mg/L)			$AUC_{0\text{-}4\ hr}$ (mg.h/L)			$Cav_{0\text{-}4h}$ (mg/L)		
Dosing Day	Dose (mg/Kg/ Day)	100	200	300	100	200	300	100	200	300	100	200	300
1	N	3	3	3	3	3	3	3	3	3	3	3	3
	Mean	2.33	2.33	2.00	23.46	26.96	36.25	73.36	83.70	112.91	18.34	20.92	28.23
	SD	1.53	1.53	0.00	7.80	4.37	5.77	18.04	10.07	17.39	4.51	2.52	4.35
	CV%	65.50	65.50	0.00	33.20	16.20	15.90	24.60	12.00	15.40	24.60	12.00	15.40
	Geometric Mean	2.00	2.00	2.00	22.57	26.73	35.94	71.74	83.28	112.03	17.93	20.82	28.01
11	N	3	3	3	3	3	3	3	3	3	3	3	3
	Mean	4.00	2.33	2.67	24.73	32.89	54.54	77.21	115.35	195.43	19.30	28.84	48.86
	SD	0.00	1.53	1.15	7.76	2.17	6.06	18.19	7.98	25.49	4.55	2.00	6.37
	CV%	0.00	65.50	43.30	31.40	6.60	11.10	23.60	6.90	13.00	23.60	6.90	13.00
	Geometric Mean	4.00	2.00	2.52	23.99	32.85	54.31	75.88	115.17	194.26	18.97	28.79	48.57

Figure IVE.1.1

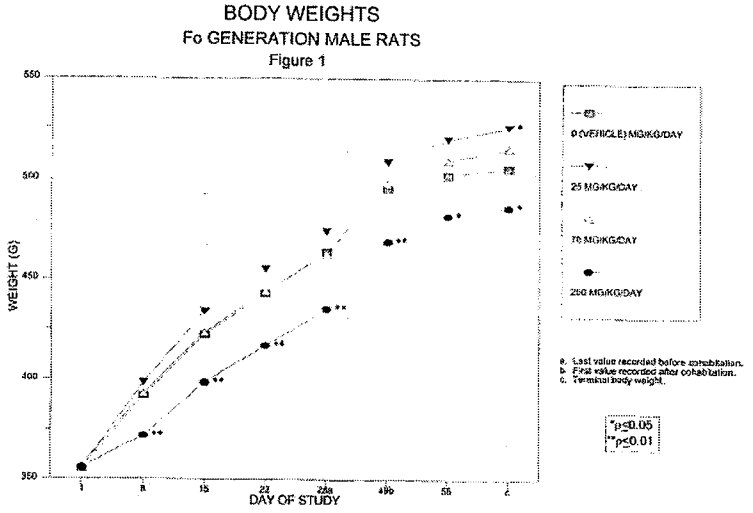

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Figure IVE.1.2

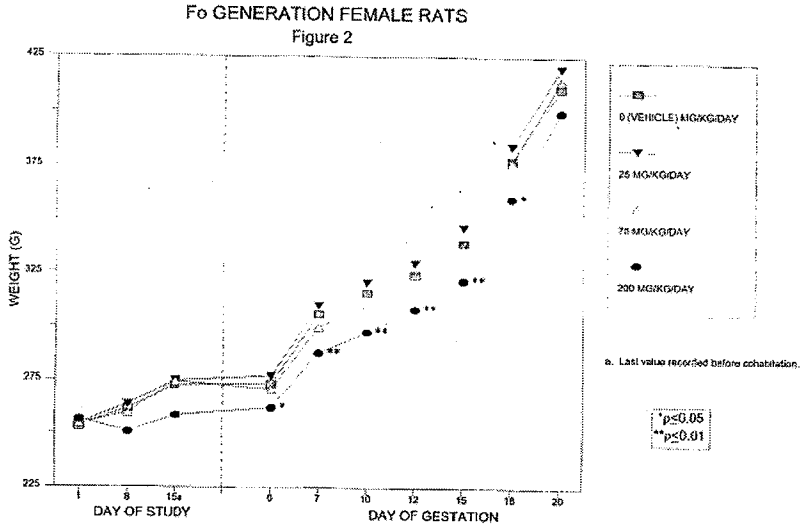

Table IVE.1.2 Body Weight Changes – F0 Generation Male Rats

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 25	III 75	IV 200
RATS TESTED	N	25	25	25	25
INCLUDED IN ANALYSES	N	25	25	25	24a
BODY WEIGHT CHANGE (G)					
DAYS 1 - 8	MEAN±S.D.	+36.8 ± 12.3	+43.6 ± 14.1	+36.4 ± 9.8	+16.3 ± 10.5**
DAYS 8 - 15	MEAN±S.D.	+30.3 ± 12.2	+35.2 ± 9.8	+30.2 ± 10.3	+26.7 ± 6.5
DAYS 15 - 22	MEAN±S.D.	+26.8 ± 8.2	+23.4 ± 10.3	+20.2 ± 6.3	+18.4 ± 7.0
DAYS 22 - 28b	MEAN±S.D.	+21.0 ± 7.8	+19.0 ± 6.9	+18.9 ± 7.4	+18.3 ± 5.0
DAYS 1 - 28b	MEAN±S.D.	+108.9± 22.3	+119.2± 22.4	+106.2± 24.2	+79.8 ± 16.1**
DAYS 49c- 56	MEAN±S.D.	+9.1 ± 11.6 [24]a	+13.2 ± 8.1	+11.1 ± 8.3	+12.5 ± 5.8 [23]a
DAYS 1 - 56	MEAN±S.D.	+147.8± 24.6 [24]a	+164.8± 28.7*	+158.3± 24.4	+125.7± 19.8** [23]a
DAY 1 - TERMINATION	MEAN±S.D.	+151.3± 25.6 [24]a	+171.0± 29.8*	+158.9± 24.2	+136.3± 19.9** [23]a

DAY(S) = DAY(S) OF STUDY
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rats that were found dead.
b. Last value recorded before cohabitation.
c. First value recorded after cohabitation.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Table IVE.1.3 Body Weight Changes – Premating– F0 Generation Female Rats

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 25	III 70	IV 200
RATS TESTED	N	25	25	25	25
BODY WEIGHT CHANGE (G)					
DAYS 1 - 8	MEAN±S.D.	+8.5 ± 5.1	+9.4 ± 12.1	+5.2 ± 6.7	-5.3 ± 10.2**
DAYS 8 - 15b	MEAN±S.D.	+11.0 ± 6.9	+11.1 ± 11.5	+14.7 ± 8.1	+7.2 ± 11.6
DAYS 1 - 15b	MEAN±S.D.	+19.4 ± 8.6	+20.4 ± 8.6	+19.9 ± 11.1	+2.0 ± 11.1**

DAYS = DAYS OF STUDY
a. Dosage occurred on day 1 of study through day 17 of presumed gestation.
b. Last value recorded before cohabitation.
** Significantly different from the vehicle control group value (p≤0.01).

Table IVE.1.4 Maternal Body Weight Changes – Gestation – F0 Generation Female Rats

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 25	III 70	IV 200
RATS TESTED	N	25	25	25	25
PREGNANT	N	23	25	23	23
EXCLUDED IN ANALYSES	N	22b	25	21b	23
MATERNAL BODY WEIGHT CHANGE (G)					
DAYS 0 - 7	MEAN±S.D.	+32.5 ± 5.8	+33.0 ± 9.0	+29.2 ± 6.5	+25.7 ± 9.3**
DAYS 7 - 10	MEAN±S.D.	+10.2 ± 4.4	+10.7 ± 4.2	+9.7 ± 9.6	+9.8 ± 6.5
DAYS 10 - 12	MEAN±S.D.	+8.1 ± 3.4	+9.8 ± 2.9	+9.8 ± 9.0	+10.5 ± 6.2
DAYS 12 - 15	MEAN±S.D.	+15.1 ± 4.1	+16.8 ± 4.7	+17.4 ± 3.4	+13.7 ± 6.9
DAYS 15 - 18	MEAN±S.D.	+38.3 ± 12.3	+39.7 ± 5.6	+40.8 ± 11.8	+38.2 ± 8.9
DAYS 0 - 18	MEAN±S.D.	+104.4± 17.6	+107.3± 15.6	+106.1± 15.4	+87.9 ± 15.6
DAYS 18 - 20	MEAN±S.D.	+33.8 ± 12.5	+35.8 ± 7.1	+37.4 ± 14.0	+39.7 ± 8.1
DAYS 0 - 20	MEAN±S.D.	+137.4± 19.5	+143.0± 18.6	+143.5± 12.1	+127.6± 18.3

DAYS = DAYS OF GESTATION
a. Dosage occurred on day 1 of study through day 17 of gestation.
b. Excludes values for rats that did not have a confirmed mating date.
** Significantly different from the vehicle control group value (p≤0.01).

Table IVE.1.5 Mating and Fertility – F0 Generation Male Rats

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 25	III 70	IV 200
RATS IN COHABITATION	N	25	25	25	24a
DAYS IN COHABITATION b	MEAN±S.D.	2.9 ± 3.0 [24]	2.6 ± 2.4	2.3 ± 1.0 [23]	2.4 ± 3.1
RATS THAT MATED c	N(%)	25(100.0)	25(100.0)	25(100.0)	24(100.0)
FERTILITY INDEX d,e	N/N (%)	23/25 (92.0)	25/25 (100.0)	23/25 (92.0)	23/24 (95.8)
RATS WITH CONFIRMED MATING DATES f	N	24	25	23	24
RATS MATING g DAYS 1-7 DAYS 8-14	N(%) N(%)	22(91.7) 2(8.3)	24(96.0) 1(4.0)	23(100.0) 0(0.0)	24(100.0) 0(0.0)
RATS PREGNANT/RATS IN COHABITATION f	N/N (%)	23/25 (92.0)	25/25 (100.0)	23/25 (92.0)	23/24 (95.8)

[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 14(18), which was found dead on day 6 of study.
b. Restricted to rats with a confirmed mating date on days 1 to 14 of cohabitation and rats that did not mate.
c. Includes only one mating for each male rat.
d. Number of pregnancies/number of rats that mated.
e. Includes only one pregnancy for each rat that impregnated more than one female rat.
f. Includes only one confirmed mating for each male rat.
g. Restricted to rats with a confirmed mating date on days 1 to 14 of cohabitation.

Table IVE.1.6 Mating and Fertility – F0 Generation Female Rats

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 25	III 70	IV 200
MATING OBSERVATIONS					
RATS IN COHABITATION	N	25	25	25	25
DAYS IN COHABITATION b	MEAN±S.D.	2.9 ± 3.0 [24]	2.6 ± 2.4	2.3 ± 1.0 [23]	2.4 ± 1.0
RATS THAT MATED	N(%)	25(100.0)	25(100.0)	25(100.0)	25(100.0)
FERTILITY INDEX c	N/N (%)	23/25 (92.0)	25/25 (100.0)	23/25 (92.0)	23/25 (92.0)
RATS WITH CONFIRMED MATING DATES	N	24	25	23	25
MATED BY FIRST MALE d DAYS 1-7 DAYS 8-14	N(%) N(%)	22(91.7) 2(8.3)	24(96.0) 1(4.0)	23(100.0) 0(0.0)	25(100.0) 0(0.0)
RATS PREGNANT/RATS IN COHABITATION	N/N (%)	23/25 (92.0)	25/25 (100.0)	23/25 (92.0)	23/25 (92.0)

[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on day 1 of study through day 17 of presumed gestation.
b. Restricted to rats with a confirmed mating date and rats that did not mate.
c. Number of pregnancies/number of rats that mated.
d. Restricted to rats with a confirmed mating date.

Table IVE.1.7 Caesarean-Sectioning Observations – F0 Generation Female Rats

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 25	III 70	IV 200
RATS TESTED	N	25	25	25	25
PREGNANT	N(%)	23(92.0)	25(100.0)	23(92.0)	23(92.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 20 OF GESTATION	N	23	25	23	23
INCLUDED IN ANALYSES	N	22b	25	21b	23
CORPORA LUTEA	MEAN±S.D.	18.3 ± 3.3	18.7 ± 2.1	18.7 ± 2.9	17.5 ± 2.5
IMPLANTATIONS	MEAN±S.D.	15.2 ± 2.8	16.1 ± 2.4	15.8 ± 1.9	15.3 ± 2.9
LITTER SIZES	MEAN±S.D.	14.4 ± 3.0	15.4 ± 2.1	15.3 ± 2.1	14.7 ± 3.0
LIVE FETUSES	N MEAN±S.D.	318 14.4 ± 3.0	386 15.4 ± 2.1	322 15.3 ± 2.1	339 14.7 ± 3.0
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.8 ± 0.9	0.6 ± 0.9	0.5 ± 0.7	0.6 ± 0.8
EARLY RESORPTIONS	N MEAN±S.D.	17 0.8 ± 0.9	16 0.6 ± 0.9	10 0.5 ± 0.7	12 0.5 ± 0.8
LATE RESORPTIONS	N MEAN±S.D.	0 0.0 ± 0.0	0 0.0 ± 0.0	1 0.0 ± 0.2	1 0.0 ± 0.2
DAMS WITH ANY RESORPTIONS	N(%)	11(50.0)	11(44.0)	9(42.8)	9(39.1)
DAMS WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE FETUSES	N(%)	22(100.0)	25(100.0)	21(100.0)	23(100.0)
PLACENTAE APPEARED NORMAL	N(%)	22(100.0)	25(100.0)	21(100.0)	23(100.0)

LITTERS WITH ONE OR MORE LIVE FETUSES	N	22	25	21	23
IMPLANTATIONS	MEAN±S.D.	15.2 ± 3.0	16.1 ± 2.4	15.8 ± 1.9	15.3 ± 2.9
LIVE FETUSES	N	318	386	322	339
	MEAN±S.D.	14.4 ± 3.0	15.4 ± 2.3	15.3 ± 2.1	14.7 ± 3.0
LIVE MALE FETUSES	N	138	201	148	178
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	41.7 ± 33.6	51.7 ± 12.4*	46.4 ± 32.9	53.0 ± 11.7*
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	3.27 ± 0.21	3.27 ± 0.41	3.30 ± 0.18	3.33 ± 0.21
MALE FETUSES	MEAN±S.D.	3.28 ± 0.21	3.36 ± 0.44	3.34 ± 0.20	3.40 ± 0.25
FEMALE FETUSES	MEAN±S.D.	3.09 ± 0.20	3.19 ± 0.40	3.25 ± 0.16*	3.25 ± 0.17*
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	5.5 ± 6.4	3.8 ± 5.0	3.4 ± 4.8	3.8 ± 6.2

a. Dosage occurred on day 1 of study through day 17 of gestation.
* Significantly different from the vehicle control group value ($p \leq 0.05$).

Table IVE.1.8 Fetal Alterations – F1 Generation Litters

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	25	70	200
LITTERS EVALUATED	N	22	25	21	23
FETUSES EVALUATED	N	318	386	322	339
LIVE	N	318	386	322	339
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%)	9(40.9)	7(28.0)	6(28.6)	11(47.8)
FETUSES WITH ANY ALTERATION OBSERVED	N(%)	12(3.8)	9(2.3)	13(4.0)	12(3.5)
% FETUSES WITH ANY ALTERATION/LITTER	MEAN±S.D.	3.7 ± 4.8	2.2 ± 4.2	5.0 ± 9.4	3.6 ± 4.2

a. Dosage occurred on day 1 of study through day 17 of gestation.

2. Oral (Stomach Tube) Developmental Toxicity Study of Harkoseride in Female Rabbits (Protocol 1108-002, report dated 12/6/00, conducted by [redacted] GLP) b(4)

a. Methods

Timed pregnant rabbits (New Zealand White; 20/grp) were treated with 0 (vehicle), 6.25, 12.5, or 25 mg/kg lacosamide by oral gavage on gestational days (GD) 6 through 18. Does were observed for viability, clinical signs, premature deliveries, and deaths. Body weights (BW) and food consumption were recorded during the dosing and postdosing period. Animals were sacrificed on GD 29 and C-sectioned. Numbers of corpora lutea was recorded and uteri were examined for pregnancy, number and distribution of implantation sites, early and late resorptions, and live and dead fetuses. All fetuses were weighed and examined for gross, soft tissue (Staples method), and skeletal alterations (alizarin red-S staining).

Strain: New Zealand White (Hra:(NZW)SPF)
Drug lot #: KK02457

Dose selection:

Doses were based on an embryofetal range-finding study in which pregnant rabbits (5/grp) were given 6.25, 12.5, 25, or 50 mg/kg on GDs 6-18. There were no deaths. Treatment-related (T-R) clinical signs included decreased motor activity and clonic and tonic extensor convulsions at the 2 highest doses (occurred throughout dosing period in some animals). BW gain was reduced during dosing at the MD and HD (BW loss over

treatment period at HD). Resorptions were increased slightly at the MD and HD (0, 0, 2.9, 2.9 % resorbed/litter in C, LD, MD, and HD), but there were no differences in numbers of live fetuses. Fetal body weights were reduced at the HD (12% compared to C). No fetal gross alterations were reported (skeletal and visceral not evaluated). TK data from this study are shown in **Tables IVE.2.1-2** below.

b. Results

i. Effects on the dam

There were no T-R deaths or abortions. One C and 1 HD doe died as the result of intubation accidents. One MD doe with a red vaginal discharge was sacrificed on GD 19, but the litter appeared intact and normal at necropsy. All other does survived to scheduled sacrifice on GD 29.

Increased incidences of limited use of the hindlimbs and rales (throughout dosing period) and single incidences of ataxia (GDs 7-8) and tonic extensor convulsion (GD16) were seen at the HD.

BW gain was only slightly and transiently decreased (NS) early during the treatment period in the HD group (**Figure IVE.2.1; Table IVE.2.**

ii. Fetal evaluations

There were no effects of treatment on C-sectioning and litter parameters (**Tables IVE.2.4-5**).

There were no apparent T-R differences in fetal external, visceral, or skeletal alterations (malformations or variations). Numbers (%) of fetuses with any alteration were 18 (12.0%), 23 (14.4%), 16 (11.0%) and 11 (8.0%) in 9 (56.2%), 12 (66.7%), 9 (50.0%) and 8 (50.0%) litters, in the C, LD, MD, and HD groups, respectively (**Table IVE.2.6**).

c. Conclusions

Treatment of pregnant rabbits with LCM (oral doses of 6.25, 12.5, or 25 mg/kg) throughout the period of organogenesis (GDs 6-18) produced no apparent adverse effects on development. The increased resorption seen at 25 mg/kg in the dose range-finding study was not replicated. Although maternal toxicity was less than usually expected in terms of BW effects, the dose range-finding study indicated that maternal convulsions were dose-limiting; therefore, the study can be considered adequate under the once daily dosing conditions.

Table IVE.2.1

Summary Statistics for Pharmacokinetic Parameters for the Different Treatment Groups on Day 1 of Dosing

Dose (mg/Kg/d)		Tmax (h)	Cmax (mg/L)	AUC(0-24h) (mg.h/L)	AUC(0-inf) (mg.h/L)	t1/2 (h)	CL/F (L/h)	Vz/F (L)
6.25	N=5							
	Mean	1.60	6.42	46.84	47.26	3.36	0.13	0.64
	SD	0.55	0.22	2.20	2.26	0.16	0.01	0.03
	Min	1.00	6.16	44.83	45.09	3.10	0.12	0.60
	Max	2.00	6.71	50.22	50.69	3.51	0.14	0.69
	CV%	34.23	3.44	4.69	4.78	4.88	4.65	5.20
	Geometric Mean	1.52	6.41	46.80	47.21	3.36	0.13	0.64
12.50	N=5							
	Mean	1.10	12.88	100.49	101.83	3.58	0.13	0.64
	SD	0.55	1.51	16.21	17.25	0.46	0.02	0.03
	Min	0.50	10.62	88.62	89.14	3.11	0.10	0.59
	Max	2.00	14.83	127.51	130.62	4.30	0.14	0.67
	CV%	49.79	11.68	16.13	16.94	12.96	14.75	4.47
	Geometric Mean	1.00	12.81	99.53	100.77	3.55	0.12	0.64
25.00	N=5							
	Mean	1.50	25.57	201.38	204.01	3.55	0.12	0.63
	SD	0.71	3.53	28.76	30.66	0.48	0.02	0.05
	Min	0.50	21.18	179.89	181.80	2.95	0.10	0.58
	Max	2.00	30.05	250.84	256.84	4.25	0.14	0.69
	CV%	47.14	13.81	14.28	15.03	13.38	13.01	7.93
	Geometric Mean	1.32	25.38	199.88	202.34	3.53	0.12	0.63
50.00	N=5							
	Mean	1.20	40.27	435.77	454.21	4.46	0.11	0.70
	SD	1.57	6.32	31.06	46.69	1.09	0.01	0.10
	Min	0.50	32.46	415.38	422.27	3.38	0.09	0.58
	Max	4.00	50.06	489.48	534.78	6.13	0.12	0.83
	CV%	130.44	15.70	7.13	10.28	24.44	9.25	14.97
	Geometric Mean	0.76	39.88	434.93	452.42	4.36	0.11	0.69

Table IVE.2.2

Summary Statistics for Pharmacokinetic Parameters for the Different Treatment Groups from Day 13 of Dosing

Dose (mg/Kg/d)		Tmax (h)	Cmax (mg/L)	AUCss(0-24h) (mg.h/L)	AUCss(0-inf) (mg.h/L)	t1/2 (h)	CLss/F (L/h/Kg)	Vzss/F (L/Kg)
6.25	N=5							
	Mean	0.50	7.13	39.62	39.78	2.93	0.16	0.67
	SD	0.00	0.37	1.74	1.78	0.13	0.01	0.01
	Min	0.50	6.54	37.45	37.55	2.75	0.15	0.65
	Max	0.50	7.55	41.86	42.08	3.07	0.17	0.68
	CV%	0.00	5.19	4.39	4.48	4.60	4.39	2.04
	Geometric Mean	0.50	7.12	39.59	39.75	2.92	0.16	0.67
12.5	N	5	5	4	4	4	4	4
	Mean	0.80	13.78	83.08	83.58	3.05	0.15	0.66
	SD	0.27	1.40	12.76	13.17	0.41	0.02	0.03
	Min	0.50	12.41	73.69	73.84	2.60	0.12	0.64
	Max	1.00	15.98	101.83	102.92	3.59	0.17	0.69
	CV%	34.23	10.17	15.36	15.76	13.39	13.61	4.62
	Geometric Mean	0.76	13.72	82.41	82.86	3.03	0.15	0.66
25.00	N=5							
	Mean	0.80	24.79	151.43	152.60	3.05	0.17	0.73
	SD	0.27	2.68	30.04	31.45	0.60	0.03	0.07
	Min	0.50	22.09	122.14	122.42	2.62	0.12	0.66
	Max	1.00	28.65	202.15	205.89	3.99	0.20	0.82
	CV%	34.23	10.81	19.84	20.61	19.62	17.24	9.32
	Geometric Mean	0.76	24.68	149.28	150.27	3.01	0.17	0.73
50.00	N=5							
	Mean	0.80	47.00	426.97	453.75	4.63	0.12	0.75
	SD	0.67	3.74	130.84	176.75	2.26	0.03	0.14
	Min	0.50	43.01	331.73	333.33	2.93	0.08	0.64
	Max	2.00	52.84	655.54	764.07	8.37	0.15	0.92
	CV%	83.85	7.96	30.64	38.95	49.05	23.09	19.15
	Geometric Mean	0.66	46.89	413.73	432.06	4.25	0.12	0.74

Figure IVE.2.1

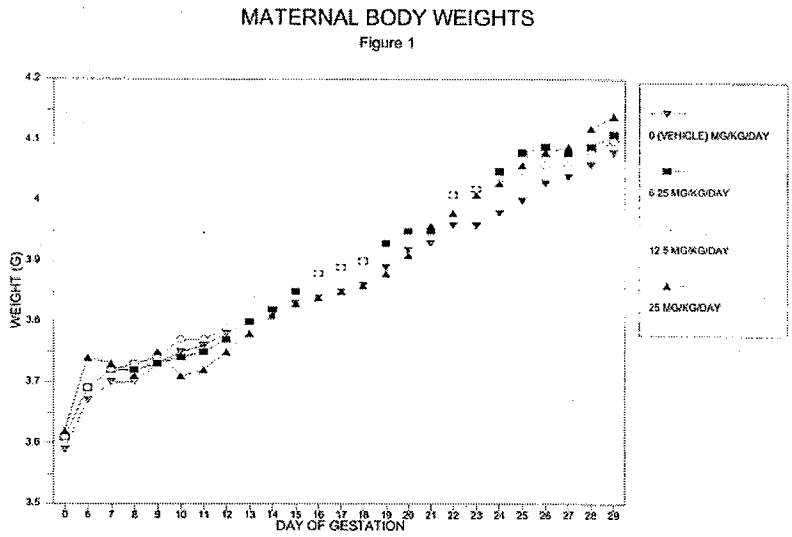

Table IVE.2.3 Maternal Body Weight Changes

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 6.25	III 12.5	IV 25
RABBITS TESTED	N	20	20	20	20
PREGNANT	N	18	18	19	17
MATERNAL BODY WEIGHT CHANGE (KG)					
DAYS 0 - 6	MEAN±S.D.	+0.08 ± 0.07	+0.08 ± 0.08	+0.08 ± 0.06	+0.12 ± 0.11
DAYS 6 - 7	MEAN±S.D.	+0.03 ± 0.04	+0.03 ± 0.04	+0.02 ± 0.06	-0.01 ± 0.05
DAYS 7 - 8	MEAN±S.D.	+0.00 ± 0.03	+0.00 ± 0.06	+0.02 ± 0.02	-0.02 ± 0.09
DAYS 8 - 9	MEAN±S.D.	+0.03 ± 0.03	+0.01 ± 0.04	+0.01 ± 0.02	+0.04 ± 0.05
DAYS 6 - 9	MEAN±S.D.	+0.06 ± 0.06	+0.04 ± 0.07	+0.05 ± 0.08	+0.01 ± 0.09
DAYS 9 - 12	MEAN±S.D.	+0.04 ± 0.05	+0.04 ± 0.04	+0.04 ± 0.05	+0.03 ± 0.07 [16]b
DAYS 12 - 15	MEAN±S.D.	+0.06 ± 0.06 [17]b	+0.07 ± 0.06	+0.07 ± 0.05	+0.08 ± 0.05 [16]b
DAYS 15 - 19	MEAN±S.D.	+0.06 ± 0.06 [17]b	+0.08 ± 0.06	+0.06 ± 0.07	+0.05 ± 0.08 [16]b
DAYS 19 - 24	MEAN±S.D.	+0.09 ± 0.07 [17]b	+0.12 ± 0.06	+0.08 ± 0.12 [18]b	+0.16 ± 0.06* [16]b
DAYS 24 - 29	MEAN±S.D.	+0.10 ± 0.13 [17]b	+0.06 ± 0.13	+0.09 ± 0.09 [18]b	+0.10 ± 0.06 [16]b
DAYS 6 - 19	MEAN±S.D.	+0.23 ± 0.13 [17]b	+0.24 ± 0.10	+0.23 ± 0.10	+0.18 ± 0.15 [16]b
DAYS 19 - 29	MEAN±S.D.	+0.19 ± 0.25 [17]b	+0.18 ± 0.14	+0.14 ± 0.17 [18]b	+0.26 ± 0.08 [16]b
DAYS 6 - 29	MEAN±S.D.	+0.42 ± 0.26 [17]b	+0.42 ± 0.17	+0.38 ± 0.18 [18]b	+0.44 ± 0.20 [16]b
DAYS 0 - 29	MEAN±S.D.	+0.50 ± 0.26 [17]b	+0.50 ± 0.23	+0.46 ± 0.19 [18]b	+0.56 ± 0.17 [16]b

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 6 through 18 of gestation.
b. Excludes values for rabbits that died or were sacrificed.
* Significantly different from the control group value ($p \leq 0.05$).

Table IVE.2.4 Caesarean-Sectioning Observations

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 6.25	III 12.5	IV 25
RABBITS TESTED	N	20	20	20	20
PREGNANT	N(%)	18(90.0)	18(90.0)	19(95.0)	17(85.0)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	17	18	16	16
CORPORA LUTEA	MEAN±S.D.	10.4 ± 1.6	9.9 ± 2.2	9.8 ± 2.9	10.2 ± 1.7 (15)b
IMPLANTATIONS	MEAN±S.D.	9.7 ± 1.0	9.2 ± 1.9	8.2 ± 2.6	9.0 ± 2.6
LITTER SIZES	MEAN±S.D.	8.8 ± 2.4	8.9 ± 1.9	8.1 ± 2.7	8.6 ± 2.2
LIVE FETUSES	N	150	160	146	138
	MEAN±S.D.	8.8 ± 2.4	8.9 ± 1.9	8.1 ± 2.7	8.6 ± 2.2
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.9 ± 2.4	0.3 ± 0.5	0.1 ± 0.3	0.4 ± 0.6
EARLY RESORPTIONS	N	13	3	0	3
	MEAN±S.D.	0.8 ± 2.4	0.2 ± 0.4	0.0 ± 0.0	0.2 ± 0.4
LATE RESORPTIONS	N	2	3	2	3
	MEAN±S.D.	0.1 ± 0.3	0.2 ± 0.4	0.1 ± 0.3	0.2 ± 0.4
DOES WITH ANY RESORPTIONS	N(%)	6(35.3)	6(33.3)	2(11.1)	5(31.2)
DOES WITH ALL CONCEPTUSES RESORBED	N(%)	1(5.9)	0(0.0)	0(0.0)	0(0.0)
DOES WITH VIABLE FETUSES	N(%)	16(94.1)	18(100.0)	18(100.0)	16(100.0)
PLACENTAE APPEARED NORMAL	N(%)	17(100.0)	18(100.0)	18(100.0)	16(100.0)

a. Dosage occurred on days 6 through 18 of gestation.
b. Excludes values that were incorrectly recorded.

Table IVE.2.5 Litter Observations (Caesarean-Delivered Fetuses)

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 6.25	III 12.5	IV 25
LITTERS WITH ONE OR MORE LIVE FETUSES	N	16	18	18	16
IMPLANTATIONS	MEAN±S.D.	9.7 ± 1.1	9.2 ± 1.9	8.2 ± 2.6	9.0 ± 2.6
LIVE FETUSES	N	150	166	146	138
	MEAN±S.D.	9.4 ± 1.0	8.9 ± 1.9	8.1 ± 2.7	8.6 ± 2.2
LIVE MALE FETUSES	N	75	78	78	70
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	50.0 ± 12.1	50.9 ± 19.1	55.7 ± 18.2	56.6 ± 14.8
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	43.84 ± 5.08	43.47 ± 4.39	45.70 ± 5.92	46.05 ± 6.18
MALE FETUSES	MEAN±S.D.	44.44 ± 5.51	43.69 ± 5.31	46.19 ± 6.17	46.43 ± 6.10
FEMALE FETUSES	MEAN±S.D.	43.13 ± 5.08	43.33 ± 4.89	44.69 ± 6.46	45.62 ± 6.74
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	3.0 ± 4.7	3.5 ± 5.1	1.5 ± 4.3	3.4 ± 5.3

a. Dosage occurred on days 6 through 18 of gestation.

Table IVE.2.6 Fetal Alterations – Summary

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 6.25	III 12.5	IV 25
LITTERS EVALUATED	N	16	18	18	16
FETUSES EVALUATED	N	160	160	146	138
LIVE	N	160	160	146	138
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%)	9(56.2)	12(66.7)	9(50.0)	8(50.0)
FETUSES WITH ANY ALTERATION OBSERVED	N(%)	18(12.0)	23(14.4)	16(12.8)	13(8.0)
% FETUSES WITH ANY ALTERATION/LITTER	MEAN±S.D.	13.2 ± 14.8	11.9 ± 13.3	9.4 ± 12.9	10.5 ± 14.4

a. Dosage occurred on days 6 through 18 of gestation.

3. Oral (Gavage) Developmental and Perinatal/Postnatal Reproduction Toxicity Study of Harkoseride (ADD 234037) in Rats, including a Postnatal Behavioral/Functional Evaluation (Protocol1108-004, report dated 12/6/00, conducted by [redacted] GLP)

b(4)

a. Methods

Presumed pregnant female rats (S-D; 25/group) were treated with oral (gavage) doses of 0 (vehicle), 25, 70, or 200 mg/kg LCM from gestational day 7 (GD 7) through day 20 of lactation (PND 20). Clinical signs and BWs were assessed in dams during the treatment period and at sacrifice, and the following reproductive endpoints were evaluated: adverse signs during parturition, duration of gestation, litter size, and pup viability at birth. Pups were counted and clinical observations were made daily during the preweaning period. Pup BWs were recorded on PNDs 1 (birth), 4, 7, 14 and 21. At the end of the 23-day postpartum period, F0 females were sacrificed, a gross necropsy was performed, and the number of implantation sites was recorded. At weaning on PND 21, 25 pups/sex/group were randomly selected for continued evaluation. Offspring BWs were recorded weekly during the postweaning period. Beginning at PND 24, 1/sex/litter were evaluated in a passive avoidance test for learning, short-term retention, and long-term retention. Females were evaluated for age of vaginal patency, beginning on PND 28, and males were evaluated for age of preputial separation, beginning on PND 39. Beginning at PND 70, 1/sex/litter were evaluated in a water-filled M-maze for coordination, swimming ability, learning and memory. At approximately PND 90, offspring were randomly assigned to mating (1:1; 21 day period). Male selected for mating were sacrificed after completion of the mating period for gross necropsy, and testes and epididymides (but not brains) were weighed. Females were sacrificed on GD 20, C-sectioned and examined for number and distribution of corpora lutea, implantation sites and live and dead fetuses. Each fetus was weighed and examined for sex and gross external alterations.

b(4)

Strain: [redacted]CD(SD)IGS BR VAF/Plus
Drug lot #: KK02457

Dose selection:

Doses were based on the rat embryofetal dose range-finding study described above. In that study (0, 100, 200, or 300 mg/kg on GD 7-17), T-R clinical signs of decreased motor activity, ataxia, impaired or lost righting reflex, urine-stained abdominal fur and excess salivation were seen at the 2 highest doses; BW gain was reduced at the MD and HD (20 and 60% compared to C over GD 7-20); resorptions were increased in the MD and HD; and fetal BWs were reduced at the HD (30% compared to C). TK data from this study are shown in **Table IVE.1.1** above.

b. Results

i. Effects on the dam

Mortality and clinical observations - Three HD dams died or were sacrificed moribund. These deaths were associated with prolonged gestation and what were considered severe clinical signs (in addition to those described for the HD group below, muscle flaccidity, bradypnea, and chromodacryorrhea). Two died or were sacrificed on GD 23 without completing parturition (litters of 13 and 16 conceptuses appeared normal); the third delivered a litter on GD 23 (litter of 15 conceptuses based on implantation sites, but only 8 delivered, 2 of these partly cannibalized) but then was sacrificed on PND 13. All other dams delivered litters and survived to scheduled sacrifice.

T-R clinical observations during gestation in HD dams included limited use of the fore- and/or hindlimbs, splayed hindlimbs, impaired proprioceptive positioning, impaired and/or lost righting reflex, decreased motor activity, ataxia and a dried red perinasal substance. One or more of these first occurred on GD 7 and the number and severity tended to increase with gestational age. It was pointed out that since the ratio of dam/fetal weight decreases with gestational age, the increased incidence and duration of clinical observations near parturition could reflect an increased maternal dose in late pregnancy. Since some HD dams continued to have adverse clinical signs on the first and/or second day of lactation, it was thought that this may have contributed to the early pup deaths in this group. However, in general, clinical signs occurred only sporadically after PND 1.

Body weight and food consumptions - Reductions in BW gain were seen at the HD (and transiently at MD) during the gestational treatment period (40% over GDs 7 to 20 at HD) compared to C (**Table IVE.3.1**). Maternal BWs tended to continue to be reduced somewhat during the lactation period at the HD (SS on PNDs 1 through 13 and 18) even though BW gain was actually increased; however there were no group differences in maternal BWs at weaning (PND 21).

Parturition and lactation parameters – Duration of gestation was D-D increased in treated groups compared to C, and the number of dams with stillborn pups was increased at all doses (**Table IVE.3.2**).

ii. Offspring evaluations

Survival - Increased numbers of stillborn (or unknown status) pups (SS at LD and HD; SS reduction in number of liveborn pups at HD) were seen in all treated litters, and increased pup mortality (PNDs 2-4) and a decreased viability index were seen at the HD (**Table IVE.3.3**). No deaths, clinical signs, or necropsy observations related to treatment were noted in the offspring postweaning.

Body Weight - Pup BWs were decreased at the HD on PND 1 and 4, and tended to be reduced throughout lactation in this group (**Table IVE.3.4**). BW and BW gains were comparable among groups during the postweaning period.

Developmental Landmarks - There were no T-R differences in sexual maturation based on day of preputial separation or vaginal patency.

Offspring Behavior - There were no clear, statistically significant differences in learning and memory in the passive avoidance and M-maze tests. However,

there was the suggestion of an effect in both (**Tables IVE.3.5-6)** . In the passive avoidance test initiated on PND 24, there was a small (NS) decrease in latency in the retention phase in HD males (11.9, 19.7, 16.7, and 8.0 sec in C, LD, MD, and HD), where the C group was lower than expected. And in the M-maze, initiated on PND 70), there was an increase in latencies during the learning phase in both males (13.7, 15.4, 14.1, and 16.2 sec) and females (12.7, 13.9, 15.2, 22 sec) and increases in latencies (8.8, 10.3, 12.6, and 13 sec) and errors (0.06, 0.17, 0.16, and 0.15 errors/trial) during the retention phase in males. There was also an increased number of animals that failed to learn in treated groups (0, 2, 1, and 1).

Offspring Reproductive Performance - There were no differences in mating performance, and C-sectioning and litter parameters in the F1 females were similar among groups.

Offspring Necropsy Observations - All necropsy observations were considered unrelated to treatment. Paired absolute epididymides weights were significantly decreased in MD males, but testes and epididymides weights (absolute and relative to BW) were comparable among other groups, including the HD.

c. Conclusions

Treatment of female rats with oral doses of 0, 25, 70, or 200 mg/kg LCM from GD 7 through PND 20 resulted in D-D increases in duration of gestation, increased numbers of stillborn pups at all doses (SS at LD and HD; SS reduction in number of liveborn pups at HD) and increased pup mortality over PNDs 2-4 at the HD. Pup BWs were decreased on PND 1 and 4 in the HD group, and tended to be reduced throughout lactation in this group. There was some suggestion of an effect on learning and memory as assessed in passive avoidance and M-maze performance, although none of the differences reached statistical significance. There were no apparent developmental effects on other endpoints evaluated, including landmarks of sexual maturation and reproductive performance. Based on the maternal toxicity observed (clinical signs and BW gain reduction), dose selection for this study was appropriate.

Table IVE.3.1 Maternal Body Weight Changes – Gestation – F0 Generation Female Rats

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	25	70	200
RATS TESTED	N	25	25	25	25
PREGNANT	N	25	25	25	24
MATERNAL BODY WEIGHT CHANGE (G)					
DAYS 0 - 7	MEAN±S.D.	+37.2 ± 7.8	+41.5 ± 6.6	+38.8 ± 7.5	+35.4 ± 7.2
DAYS 7 - 10	MEAN±S.D.	+16.3 ± 4.4	+15.0 ± 4.5	+7.2 ± 7.1**	-4.3 ± 10.9**
DAYS 10 - 12	MEAN±S.D.	+11.8 ± 4.7	+12.5 ± 4.5	+12.6 ± 4.2	+10.6 ± 9.3
DAYS 12 - 15	MEAN±S.D.	+17.6 ± 5.4	+17.8 ± 5.2	+16.2 ± 6.3	+13.6 ± 8.5
DAYS 15 - 18	MEAN±S.D.	+11.8 ± 8.9	+12.1 ± 6.8	+14.9 ± 5.8	+21.1 ± 14.6
DAYS 18 - 20	MEAN±S.D.	+28.5 ± 6.4	+27.4 ± 8.4	+28.8 ± 5.8	+19.2 ± 12.5*
DAYS 7 - 20	MEAN±S.D.	+106.2± 16.3	+104.8± 15.7	+99.8 ± 15.1	+63.2 ± 28.7*
DAYS 0 - 20	MEAN±S.D.	+143.4± 20.8	+146.3± 17.0	+138.6± 17.3	+97.7± 30.8**

DAYS = DAYS OF GESTATION
a. Dosage occurred on day 7 of gestation through day 20 of lactation.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Table IVE.3.2 Natural Delivery Observations – F0 Generation Female Rats

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 25	III 75	IV 200
RATS ASSIGNED TO NATURAL DELIVERY	N	25	25	25	25
PREGNANT	N(%)	25(100.0)	25(100.0)	25(100.0)	24(96.0)
DELIVERED LITTERS	N(%)	25(100.0)	25(100.0)	25(100.0)	22(93.7)
DURATION OF GESTATION b	MEAN±S.D.	22.4 ± 0.5	22.6 ± 0.6**	22.9 ± 0.3**	23.0 ± 0.4**
DAMS DELIVERING:					
DG 22	N(%)	14(56.0)	5(20.0)**	3(12.0)**	1(4.5)**
DG 23	N(%)	11(44.0)	20(80.0)**	22(88.0)**	19(86.4)**
DG 24	N(%)	0(0.0)	0(0.0)	0(0.0)	2(9.1)
IMPLANTATION SITES	N	349	354	375	320
PER DELIVERED LITTER	MEAN±S.D.	14.0 ± 2.6	14.2 ± 2.5	15.0 ± 1.3	14.5 ± 2.2
DAMS WITH STILLBORN PUPS	N(%)	0(0.0)	4(16.0)	2(8.0)	2(9.1)
DAMS WITH NO LIVEBORN PUPS	N	0	0	0	0
GESTATION INDEX c	%	100.0	100.0	100.0	91.7
	N/N	25/ 25	25/ 25	25/ 25	22/ 24
DAMS WITH ALL PUPS DYING DAYS 1-4 POSTPARTUM	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.5)
DAMS WITH ALL PUPS DYING DAYS 5-21 POSTPARTUM	N	0	0	0	0

DG = DAY OF GESTATION
a. Dosage occurred on day 7 of gestation through day 20 of lactation.
b. Calculated as the time (in days) elapsed between confirmed mating (arbitrarily defined as 0 day) and the time (in days) the first pup was delivered.
c. Number of rats with live offspring/number of pregnant rats.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Table IVE.3.3 Litter Observations (Naturally Delivered Pups) – F1 Generation Litters

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 25	III 75	IV 200
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	25	25	25	22
PUPS DELIVERED (TOTAL)	N	334	334	353	286
	MEAN±S.D.	13.4 ± 2.5	13.4 ± 2.8	14.1 ± 1.9	13.0 ± 3.0
LIVEBORN	MEAN±S.D.	13.4 ± 2.5	13.1 ± 2.8	14.0 ± 1.9	12.4 ± 3.2
	N(%)	334(100.0)	328(98.2)	351(99.4)	274(95.8)**
STILLBORN	MEAN±S.D.	0.0 ± 0.0	0.2 ± 0.5	0.1 ± 0.3	0.3 ± 1.1
	N(%)	0(0.0)	5(1.5)**	2(0.6)	7(2.4)**
UNKNOWN VITAL STATUS	N	0	1	0	5
PUPS FOUND DEAD OR PRESUMED CANNIBALIZED					
DAY 1	N/N(%)	0/334(0.0)	1/328(0.3)	2/351(0.6)	2/274(0.7)
DAYS 2- 4	N/N(%)	1/334(0.3)	2/327(0.6)	2/349(0.6)	16/272(5.9)**
DAYS 5- 7	N/N(%)	1/333(0.3)	1/325(0.3)	0/347(0.0)	1/256(0.4)
DAYS 8-14	N/N(%)	1/330(0.3)	0/324(0.0)	0/347(0.0)	1/255(0.4)
DAYS 15-21	N/N(%)	0/329(0.0)	0/324(0.0)	0/347(0.0)	0/254(0.0)
VIABILITY INDEX b	%	98.5	98.8	98.9	92.3**
	N/N	329/334	324/328	347/351	254/274
LACTATION INDEX c	%	100.0	100.0	100.0	100.0
	N/N	329/329	324/324	347/347	254/254

DAY(S) = DAY(S) POSTPARTUM
a. Dosage occurred on day 7 of gestation through day 20 of lactation.
b. Number of live pups on day 7 postpartum/number of liveborn pups on day 1 postpartum.
c. Number of live pups on day 21 (weaning) postpartum/number of live pups on day 7 postpartum.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Table IVE.3.4 Litter Observations (Naturally Delivered Pups) – F1 Generation Litters

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	25	70	200
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	25	25	25	22
PUP WEIGHT/LITTER (GRAMS)					
DAY 1	MEAN±S.D.	6.6 ± 0.4	6.6 ± 0.6	6.6 ± 0.4	6.2 ± 0.6**
DAY 4	MEAN±S.D.	9.0 ± 0.9	9.2 ± 1.0	8.7 ± 1.0	8.1 ± 1.3** [21]b
DAY 7	MEAN±S.D.	12.3 ± 1.9	12.6 ± 2.2	12.0 ± 1.5	11.4 ± 2.1 [21]b
DAY 14	MEAN±S.D.	23.9 ± 4.3 [24]c	24.7 ± 3.9	22.6 ± 3.1	23.9 ± 4.5 [20]b,d
DAY 21	MEAN±S.D.	37.2 ± 6.4	37.5 ± 6.6	34.0 ± 4.5	33.7 ± 7.6 [20]b,d

DAY = DAY POSTPARTUM
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on day 7 of gestation through day 20 of lactation.
b. Excludes values for litter 14092, which had no surviving pups on day 4 of lactation.
c. Excludes values that were not recorded.
d. Excludes values for litter 14081; the dam was scribunit sacrificed on day 13 of lactation.
** Significantly different from the control group value ($p \leq 0.01$).

Table IVE.3.5 Passive Avoidance Performance – F1 Generation Litters

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	25	70	200
MALE RATS					
SESSION 1a	N	25	25	25	20
TRIALS TO CRITERION	MEAN±S.D.	4.3 ± 0.9	4.2 ± 1.3	4.8 ± 2.3	4.0 ± 0.8
LATENCY TRIAL 1b	MEAN±S.D.	8.2 ± 4.8	8.5 ± 6.7	12.1 ± 14.4	10.9 ± 13.0
LATENCY TRIAL 2b	MEAN±S.D.	28.4 ± 21.8	30.0 ± 23.4	18.4 ± 15.6	30.2 ± 22.3
FAILED TO LEARN c	N(%)	0(0.0)	0(0.0)	1(4.0)	0(0.0)
SESSION 2a	N	25	25	24	20
TRIALS TO CRITERION	MEAN±S.D.	3.3 ± 0.5	3.2 ± 1.1	3.0 ± 0.6	3.3 ± 0.8
LATENCY TRIAL 1b	MEAN±S.D.	11.9 ± 13.8	19.7 ± 22.1	16.7 ± 18.0	8.0 ± 11.1
FEMALE RATS					
SESSION 1a	N	25	25	25	20
TRIALS TO CRITERION	MEAN±S.D.	4.4 ± 1.1	4.4 ± 1.5	4.1 ± 0.6	4.1 ± 0.6
LATENCY TRIAL 1b	MEAN±S.D.	6.5 ± 6.0	6.0 ± 4.8	8.4 ± 4.8	7.4 ± 5.2
LATENCY TRIAL 2b	MEAN±S.D.	23.8 ± 21.6	27.8 ± 21.9	25.1 ± 21.6	25.8 ± 22.0
FAILED TO LEARN c	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
SESSION 2a	N	25	25	25	20
TRIALS TO CRITERION	MEAN±S.D.	2.9 ± 0.3	2.8 ± 0.4	3.0 ± 0.4	2.8 ± 0.4
LATENCY TRIAL 1b	MEAN±S.D.	20.7 ± 18.4	22.0 ± 20.0	9.2 ± 12.0	19.1 ± 20.8

a. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.
b. The latency was recorded in seconds.
c. Number of rats that did not meet the criterion in Session 1 (Learning Phase); Session 2 (Retention Phase) values for these rats were excluded from group averages and statistical analyses.

Table IVE.3.6 Watermaze Performance – FI Generation Rats

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	25	70	200
MALE RATS					
SESSION 1a	N	24b	21b	23b	20
TRIALS TO CRITERION	MEAN±S.D.	8.7 ± 2.3	8.3 ± 2.6	8.1 ± 2.6	8.4 ± 2.7
ERRORS PER TRIAL	MEAN±S.D.	0.33 ± 0.15	0.36 ± 0.24	0.32 ± 0.19	0.35 ± 0.32
LATENCY TRIAL 2c	MEAN±S.D.	13.7 ± 10.6	15.4 ± 10.1	14.1 ± 11.8	16.2 ± 15.7
FAILED TO LEARN d	N(%)	0	1(4.3)	1(4.3)	0
SESSION 2a	N	24b	22b	22b	20
TRIALS TO CRITERION	MEAN±S.D.	6.2 ± 2.0	6.4 ± 1.8	6.7 ± 1.7	6.6 ± 2.5
ERRORS PER TRIAL	MEAN±S.D.	0.06 ± 0.14	0.17 ± 0.20	0.16 ± 0.18	0.15 ± 0.20
LATENCY TRIAL 1c	MEAN±S.D.	8.8 ± 4.2	16.3 ± 7.8	12.6 ± 6.9	13.0 ± 7.3
FEMALE RATS					
SESSION 1a	N	25	25	25	20
TRIALS TO CRITERION	MEAN±S.D.	9.2 ± 2.5	9.3 ± 2.5	8.7 ± 2.4	8.9 ± 2.8
ERRORS PER TRIAL	MEAN±S.D.	0.49 ± 0.32	0.43 ± 0.24	0.52 ± 0.40	0.47 ± 0.25
LATENCY TRIAL 2c	MEAN±S.D.	12.7 ± 7.6	13.9 ± 6.7	15.2 ± 11.7	22.0 ± 16.8
FAILED TO LEARN d	N(%)	0(0.0)	1(4.0)	0(0.0)	1(5.0)
SESSION 2a	N	25	24	25	19
TRIALS TO CRITERION	MEAN±S.D.	6.2 ± 2.2	6.4 ± 1.7	6.8 ± 1.5	6.9 ± 2.9
ERRORS PER TRIAL	MEAN±S.D.	0.12 ± 0.16	0.14 ± 0.15	0.11 ± 0.13	0.18 ± 0.22
LATENCY TRIAL 1c	MEAN±S.D.	13.6 ± 11.2	14.9 ± 10.6	13.4 ± 7.6	13.9 ± 9.3

a. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.
b. Excludes values for rats that did not have values recorded for Trial 1 in Session 1.
c. The latency was recorded in seconds.
d. Number of rats that did not meet the criterion in Session 1 (Learning Phase); Session 2 (Retention Phase) values for these rats were excluded from group averages and statistical analyses.

4. 6-Week Subchronic Toxicity Study of SPM 927 by Oral Administration to Juvenile CD Rats (Report No. 18602/04, dated 1/11/06, Conducted By [redacted] GLP) b(4)

a. Methods

Young rats (10/sex/dose main, 6/sex/grp recovery, 10/sex/grp reproduction from 15 dams/group; whole litter design) were given 0 (0.5% HPMC vehicle), 30, 90, or 180 mg/kg LCM (10 mL/kg) by gavage once daily for 42 days beginning at 7 days of age (study Day 1). Survival, clinical signs, body weights, clinical pathology, auditory function, ophthalmology, developmental landmarks (lower incisor eruption, eye opening, preputial separation, and vaginal opening), neurobehavioral function (FOB, open field, Morris water maze, grip strength, locomotor activity), reproductive performance, and gross and microscopic pathology were evaluated. TK determinations were made on Days 1 and 42 in groups of satellite animals (28/sex/grp)

Strain: [redacted] CD
Drug Lot #: WE 11837

Dose selection was based on a 6-week dose-range-finding study in juvenile rats in which oral (gavage) doses of 30, 100, or 300 mg/kg were administered from day 7 to day 48 of age (groups 1-4, 5/sex/grp). Another group (5) was given 100 mg/kg for 35 days starting on PND 14. Reduced motility and reduced body temperature were noted at the 2 highest doses, and body weight gain was D-D reduced at these doses (40% over total treatment period at HD). In addition, tremor, piloerection and a thickened abdomen were seen on single test days at the HD. A corresponding decrease in food consumption was also seen. Decreased absolute brain weights were seen in MD and HD groups. At the HD, 1 of 5 males and 2 of 5 females died prematurely during the treatment period after weaning. Increased mortality was also observed before weaning in this group. There were no T-R histopathological changes in the brains of animals examined on PND 56 or

those that died early (immersion fixed, H&E stained sections of cerebrum, cerebellum, brain stem, and forebrain from groups 1, 4, and 5). TK showed that levels of parent decreased over the course of treatment (**Table IVE.4.1**), with 2-4-fold higher Cmax and 3-5-fold higher AUC values on treatment day 1 compared to treatment day 42, respectively. Compared to the 13-week rat toxicity study in adults, Cmax and AUC on treatment day 42 were generally comparable, except for AUC at the MD and HD, which were still about 2-fold higher in the juvenile animals. The opposite trend was found for the O-desmethyl metabolite (SPM 12809), for which Cmax and AUC increased with repeated administration. The relative exposure to SPM 12809 was less than 10% after the first dose, and ranged from 18 to 61% with repeated dosing. Comparable data were obtained in a previous repeated dose study in the adult rat. There were no clear sex differences. Because of increased mortality at the HD, doses of 30, 90 and 180 mg/kg were selected for the main study. The same doses were used in the 6-month rat toxicity study.

b. Results

i. Mortality, clinical signs

The number of pups that died during the preweaning treatment period appeared to be increased somewhat at the HD (**Table IVE.4.2**). (These animals were replaced with littermates.) None of the main study or recovery animals died prematurely after weaning. The single death of a MD male from the reproduction subgroup (no. 127, found dead on test day 35) was considered incidental.

There were no T-R clinical signs of toxicity.

ii. Body weight

BW gain was decreased in HD males and females (30 and 23% compared to C) during the lactational treatment period (**Table IVE.4.3**). After weaning, body weight gain was increased compared to C, but the BW deficits persisted in males until the end of the treatment period (Day 49) and in females until the end of the recovery period (Day 76/77). BW at weaning was approx 20% below C in both sexes.

iii. Developmental Landmarks

There was a significant delay (approx. 2.5 days, SS) in vaginal opening in HD females (**Table IVE.4.4**). Although this corresponded to the reduced body weight gain in this group, the degree of delay was greater than would be expected if the effect was strictly secondary.

iv. Clinical Pathology, Ophthalmologic and Auditory Examinations

At the end of treatment (PND 49), cholesterol, ALT, and ALP were increased slightly in MD and HD animals. These changes were no longer seen after the recovery period. There were no notable changes in hematological or urinalysis parameters. Ophthalmologic and auditory examinations did not reveal any T-R effects.

v. Developmental Neurotoxicity Testing (Recovery and Reproduction animals)

Open field testing performed 8 days after cessation of dosing (PND 57) showed a D-D decreased latency to move from the center sector for MD and HD males and

females (SS in HD females) and decreased number of sectors entered in MD and HD males (SS at both doses; **Table IVE.4.5**.

In the Morris water maze conducted starting on PND 57, an apparent learning deficit was seen in HD males on the first 2 days of learning and in HD females on the first day of learning (SS in males on Learning Day 2). These animals eventually reached latencies comparable to controls in the learning phase. HD females also showed a deficit in the memory phase of the test, having SS greater latencies to find the platform (**Table IVE.4.6**).

Locomotor activity monitoring on PND 57 showed a slight tendency toward increased activity in treated females, although SS was never reached.

The observational neurological screening tests performed at the end of the treatment period (on PND 48) or at the end of the recovery period (PNDs 74, 75, 76 or 77) found an increase in abnormal response in the positive geotropism-test at both times in treated groups, primarily at the MD and HD, although the number of animals displaying the abnormal response was low (10%).

vi. Reproductive Performance (13th week of age, 1:1 non-sibling mating)

There was no effect on female pre-coital times or fertility index (all 100%).

There was no effect on male fertility index. Examination of sperm number, viability and morphology did not show any T-R differences. There were no T-R differences in male reproductive organ weights.

Reproductive outcome parameters at laparotomy (number of corpora lutea, implantation sites, total, early and late resorptions, placentas and live fetuses as well as the resorption rates and the pre- and post-implantation loss) were generally similar among groups. However, small non-D-R increases in pre-implantation loss (Mean %: 1.6, 8.5, 6.7, 2.3) and early resorptions (total: 5, 9,14, 9) were seen in treated groups, reaching SS at the LD and MD.

vii. Pathology

Decreased absolute brain wts (SS in HD females) were found at the end of the treatment period and after the recovery period (**Table IVE.4.7-8**). Relative brain wts were also D-D decreased (NS) in females at the end of treatment. The histological examination (all C and HD from main study and recovery groups) did not reveal any apparent T-R morphological lesions in the brain (perfusion-fixed, H.& E.-stained paraffin sections of the brain stem, caudate nucleus, cerebellum, cerebrum with cortex frontalis and cortex parietalis, retrosplenial cortex and cingulate cortex), gonads (H.& E.-stained paraffin sections of the epididymis, prostate, seminal vesicle, testis, cervix, ovary, oviduct, vagina, uterus), heart (H.& E.-stained paraffin sections of the apex, left and right atrium and ventricle as well as the septum, scarlet R staining) or in any other organ or tissue.

viii. Plasma drug levels

TK data summarized in **Table IVE.4.9** below show exposures that were lower than expected based on those in the preliminary study but that exhibit the same pattern of being higher than adult levels initially then declining over the treatment period. Compared to the 6-mo rat toxicity study using the same doses, systemic exposures at the MD and HD were up to approx. 2- and 4-fold higher based on

Cmax and up to approx. 3- and 5-fold higher based on AUC, respectively, after the first dose on PND 7. At the end of the 6-week treatment period (PND 48), exposures were comparable to those in adults.

b. Conclusions

Administration of LCM to young rats for 6 weeks beginning on PND 7 at doses of 30, 90, or 180 mg/kg decreased BW gain during lactation (HD), delayed sexual maturation in females (HD), decreased absolute and relative brain weights (D-D), and produced long-term neurobehavioral changes (altered open field performance [MD, HD], deficits in Morris Maze learning and memory[HD]).

Table IVE.4.1. TK parameters for SPM 927 measured in juvenile rats in preliminary study

Group	Day	Dose	Males			Females		
		[mg/kg/day]	C_{max} [µg/mL]	t_{max} [h]	AUC_{last} [h µg/mL]	C_{max} [µg/mL]	t_{max} [h]	AUC_{last} [h µg/mL]
2	1	30	18.20	2	243	19.55	0.5[a)]	244
	15		13.98	0.5[a)]	122	14.57	2	134
	42		10.40	2	67.0	10.60	0.5[a)]	59.2
3	1	100	59.65	2	764	64.98	2	1004
	15		23.13	2	332	27.23	0.5[a)]	340
	42		27.79	0.5[a)]	302	32.21	0.5[a)]	358
4	1	300	181.70	8	3183	193.90	2	3386
	15		54.36	2	726	66.31	0.5[a)]	903
	42		48.13	2	627	NA	NA	NA

NA denotes not applicable due to premature deaths
a) first sampling time

Table IVE.4.2 Mortality between lactation days 7 and 21

Group	Number of prematurely deceased pups (between lactation day 7 and 21)	
	male pups	female pups
1	6	4
2	9	4
3	5	5
4	10	7

Table IVE.4.3 Body Weight Gain in Juvenile Rats

	Group 1 Control		Group 2 30 mg/kg		Group 3 90 mg/kg		Group 4 180 mg/kg	
	m	f	m	f	m	f	m	f
				Lactation day				
				Body Weight Gain (%)				
TD 7-21								
Mean:	263.87	266.34	268.01	271.13	259.15	245.63	181.03	204.29
SD:	24.43	19.77	55.24	30.15	29.44	26.56	64.74	46.67
TD 21-49								
Mean:	365.59	245.08	354.78	233.28	392.99	273.81	434.51	307.00
SD:	38.87	49.35	49.53	42.89	58.21	65.29	121.49	66.94

TD 7 = first day of dosing

Table IVE.4.4 Morphological Landmarks in Juvenile Rats

Parameter	Group 1 Control	Group 2 30 mg/kg	Group 3 90 mg/kg	Group 4 180 mg/kg
		females		
VAGINAL OPENING				
Day of life:				
Mean:	30.9	31.3	30.9	33.4
SD:	0.7	1.6	0.7	2.1
t:	-	ns	ns	3.604 **
Day after conception				
Mean:	52.7	53.1	52.4	55.0
SD:	0.7	1.6	1.0	1.9
t:	-	ns	ns	3.535 **

Table IVE.4.5 Open field performance in juvenile rats

Parameter		Group 1 Control	Group 2 30 mg/kg	Group 3 90 mg/kg	Group 4 180 mg/kg
TD 57			males		
LATENCY (sec)					
	Mean:	3.9	2.7	1.7	1.9
	SD:	3.7	3.5	1.8	3.6
	t:	-	ns	ns	ns
SECTORS ENTERED (number of events per animal)					
	Mean:	52.5	47.6	26.6	26.9
	SD:	29.3	22.5	21.1	19.8
	t:	-	ns	3.067 **	3.081 **
TD 57			females		
LATENCY (sec)					
	Mean:	3.3	2.7	1.3	0.5
	SD:	2.9	3.6	1.6	1.1
	t:	-	ns	ns	3.514 **
SECTORS ENTERED (number of events per animal)					
	Mean:	76.2	61.4	54.8	62.1
	SD:	33.7	22.2	17.2	30.2
	t:	-	ns	ns	ns

Table IVE.4.6 Morris Maze performance in juvenile rats

	Learning - 4 trials per day for 4 test days - (Age of animals: 57 ± 1 to 60 ± 1 days)			
	Time to escape onto the platform (sec)			
	males			
	Learning Day 2 (Age: 58 ± 1 days)			
	1.	2.	3.	4.
Group 1: Control				
Mean:	30.6	19.5	22.8	13.5
SD:	24.6	18.6	24.8	11.3
t:	-	-	-	-
Group 2: 30 mg/kg				
Mean:	49.1	21.3	16.7	23.9
SD:	30.0	18.6	13.0	20.8
t:	ns	ns	ns	ns
Group 3: 90 mg/kg				
Mean:	30.6	22.9	27.9	16.5
SD:	23.1	23.2	26.7	11.9
t:	ns	ns	ns	ns
Group 4: 180 mg/kg				
Mean:	48.6	38.3	34.3	28.5
SD:	28.7	33.0	25.1	15.0
t:	ns	ns	ns	2.778 **

** : ($p \leq 0.01$)

females

Learning Day 1 (Age: 57 ± 1 days)

	1.	2.	3.	4.
Group 1: Control				
Mean:	82.4	60.8	49.4	38.1
SD:	20.8	35.1	34.8	32.9
t:	-	-	-	-
Group 2: 30 mg/kg				
Mean:	85.0	61.6	50.8	35.3
SD:	16.0	30.2	35.1	31.7
t:	ns	ns	ns	ns
Group 3: 90 mg/kg				
Mean:	81.5	62.9	25.9	35.6
SD:	21.5	32.5	21.8	29.0
t:	ns	ns	ns	ns
Group 4: 180 mg/kg				
Mean:	84.7	65.6	52.8	52.3
SD:	21.3	33.6	36.4	36.0
t:	ns	ns	ns	ns

** : ($p \leq 0.01$)

APPEARS THIS WAY
ON ORIGINAL

Memory
- 1 trial 4 days after the last learning day -
(Age of animals: 64 ± 1 days)

Time to escape onto the platform (sec)

	Group 1 Control	Group 2 30 mg/kg	Group 3 90 mg/kg	Group 4 180 mg/kg
MALES				
Mean:	13.3	17.4	21.3	12.9
SD:	18.8	15.0	24.3	13.9
t:	-	ns	ns	ns
FEMALES				
Mean:	11.1	17.9	13.4	32.2
SD:	10.6	12.7	11.2	28.4
t:	-	ns	ns	2.782 **

** : ($p \leq 0.01$)

APPEARS THIS WAY
ON ORIGINAL

Table IVE.4.7 Mean brain weight in juvenile rats at the end of treatment (TD 49)

	Absolute (gm)		Relative (gm/kg bw)	
	Male	Female	Male	Female
Control	2.116	2.056	8.802	12.443
30 mg/kg	2.043	1.950	8.958	11.947
90 mg/kg	2.068	1.954	8.625	11.704
180 mg/kg	1.989	1.875**	9.261	11.333

** p≤0.01

Table IVE.4.8 Mean brain weight in juvenile rats at the end of the recovery period (TD 76/77/78)

	Absolute (gm)		Relative (gm/kg bw)	
	Male	Female	Male	Female
Control	2.275	2.087	5.770	8.435
30 mg/kg	2.210	2.022	5.927	8.943
90 mg/kg	2.313	1.952	5.093	8.313
180 mg/kg	2.163	1.968	6.290	9.102

Table IVE.4.9 TK parameters for SPM 927 (lacosamide) in juvenile rats

Relationship to dose level		SPM 927					
Day		Group 2		Group 3		Group 4	
	Dose [mg/kg/day]	30		90		180	
	Fold increase				3		6
		Males	Females	Males	Females	Males	Females
1	C_{max} [µg/mL]	16.49	17.12	53.21	47.34	140.34	141.56
	Fold increase			**3.2**	**2.8**	**8.5**	**8.3**
	AUC_{0-8h} [h µg/mL]	113	128	312	204	965	606
	Fold increase			**2.8**	**1.6**	**8.6**	**4.7**
42	C_{max} [µg/mL]	11.14	13.24	29.87	21.09	41.69	42.32
	Fold increase			**2.7**	**1.6**	**3.7**	**3.2**
	AUC_{0-8h} [h µg/mL]	68.8	78.7	180	147	268	290
	Fold increase			**2.6**	**1.9**	**3.9**	**3.7**

5. 6-Week Dose-Range-Finding Study for a 33-Week Chronic Toxicity Study Of SPM 927 by Repeated Oral Administration to Juvenile Beagle Dogs- Age at Start of Administration: 8 Weeks - (Report No. 20614/06, Dated 7/3/07, Conducted By GLP) b(4)

a. Methods

Juvenile Beagle dogs (2/sex/group)were given LCM at oral (gel capsule) doses of 5, 10, or 25 mg/kg for 6 weeks starting at 8 weeks of age in order to select doses for a 33-week study. Observations consisted of clinical signs, body weight, food and water consumption, growth parameters, bone mineral density, reflex and neurofunctional testing, ECG, clinical chemistry, TK determinations, ophthalmology, organ weights, and macroscopic and histomorphological examinations.

Species/Breed: Dog / Beagle
Drug Batch #: 0411180004

Dose selection was based on the general toxicology data, specifically the chronic toxicity study in adult Beagle dogs. In this study, the NOAEL was 10 mg/kg, and the HD was 20/25 mg/kg.

b. Results

i. Mortality and Clinical signs

There were no early deaths. Staggering gait, tremor, lateral position, "slight" tonic or clonic-tonic convulsions (2 males, 1 female), and emesis were observed in most HD dogs during the first 2 weeks of treatment. Symptoms occurred between 5 minutes and 2 hours after administration and lasted up to 2 hours. In addition, the abdominal wall was indurated in most HD animals, and 1 of 2 HD males had wide opened eyes on test day 2. Clinical observation prior to dosing revealed no changes for any group.

ii. Body weight, growth and bone parameters

There were no T-R effects on BW, other growth parameters (tibia and femur length and the shoulder height), or on bone mineral content, area, or density at necropsy.

iii. Electrocardiographic examinations

There were no clearly T-R changes in ECG or blood pressure parameters following dosing on days 1, 13, and 42. 2nd degree AV-block was recorded in some treated animals at various times without any clear relationship to dosing (ie, before start of treatment, predose, and postdose) on test days 1 and 13 but not on test day 42. This was considered to be a spontaneous finding.

iv. Clinical Pathology, Ophthalmologic Examination

There were no apparent T-R effects on hematological, clinical chemistry or urinalysis parameters or on the eye.

v. Developmental Neurotoxicity Testing

Reflex testing performed during test week 6 prior to dosing did not indicate any T-R effects.

Neurological screening tests (sensory function and reactivity to auditory, visual and proprioceptive stimuli, based on Gad [Gad SC Arch Toxicol Suppl 5:256-266, 1982]) performed during test week 6 prior to dosing did not reveal any T-R differences.

vi. Anatomic pathology

At necropsy, no T-R macroscopic or organ weight changes were observed.

There were no T-R histopathological findings. The examination included perfusion fixation and an expanded examination of the brain (H.& E.-stained paraffin sections of the cerebrum, cerebellum, brain stem, pons cerebri, cortex frontalis, singular cortex, retrosplenial cortex, cortex parietalis, caudate nucleus, thalamus, hippocampus, substantia nigra and medulla oblongata I and II) and heart (H.& E.- stained paraffin sections of the left and right ventricle, left and right atrium, cross and longitudinal section of the septum with His bundle, AV node region and sinus node region; scarlet R staining) as well as a full panel of other organ and tissues.

Bone mineral content, area, and density determinations did not show any effects of treatment.

vii. Toxicokinetics

TK data summarized in **Table IVE.5.1** show relatively low exposures to parent and the major metabolite compared to those in juvenile rats or humans at MRD. There were no pronounced sex or age effects; levels were somewhat lower than in adult dogs in the chronic toxicity study (at 13 weeks, AUC: 124 and 129 ug.h/ml, Cmax: 26.7 and 25.3 ug/ml in M and F at HD). High variability was observed between animals and levels generally decreased between days 1 and 42, particularly at the LD and MD.

c. Conclusions

When LCM was given to young dogs for 6 weeks beginning at 8 weeks of age at doses of 5, 10, or 25 mg/kg, clinical signs, including convulsions, were seen at the HD during the first 2 weeks of treatment, but there were no apparent effects on body weight or other growth parameters, reflex and neurological testing, ECG, clinical chemistry, ophthalmology, or pathology.

Table IVE.5.1 TK parameters for SPM 927 (lacosamide) and SPM 12809 in juvenile dogs

Group	Day	Dose	Males			Females		
		[mg/kg/day]	C_{max} [µg/mL]	t_{max} [h]	AUC_{last} [h µg/mL]	C_{max} [µg/mL]	t_{max} [h]	AUC_{last} [h µg/mL]
SPM 927								
2	1	5	7.63	2	43.2	7.91	1.5	41.0
	42		5.02	1.5	19.8	3.36	1.5	11.7
3	1	10	11.8	1.5	54.9	11.2	1.5	44.9
	42		8.52	2.5	27.8	7.58	2	27.3
4	1	25	22.9	1.5	90.6	23.0	1	89.4
	42		19.8	1.5	77.5	24.5	1.5	101
SPM 12809								
2	1	5	0.59	4	3.35	0.69	5	3.38
	42		1.07	4	5.57	0.71	5	3.61
3	1	10	1.78	4	10.5	2.43	4	13.4
	42		2.02	3	9.55	1.74	4	8.69
4	1	25	6.60	4	36.0	5.77	4	31.7
	42		5.26	3	27.7	4.69	3	26.4

AUC (based on data collected up to 8 hrs pd)

V. SUMMARY AND EVALUATION

(ADME and General toxicology portions of this NDA were reviewed by Belinda Hayes – DAARP)

Pharmacology

Lacosamide (LCM, SPM 927, (R)-2-acetamido-*N*-benzyl-3-methoxypropionamide; previously referred to as harkoseride or ADD 234037) is a member of a series of functionalized amino acids that were specifically synthesized as anticonvulsive drug candidates. LCM demonstrated anticonvulsant effectiveness in different rodent seizure models and antinociceptive potential in experimental animal models of neuropathic pain. The mechanism of action of LCM is not fully understood, but has been proposed to involve its ability to enhance the slow inactivation of voltage-gated sodium channels (VGSC) and to interact with the collapsin response mediator protein-2 (CRMP-2).

In electrophysiological experiments, LCM, at what were considered clinically relevant concentrations (32-100 µM; concentration range associated with clinical doses: 10 to 60 µM), reduced VGSC-mediated sodium currents in neuroblastoma cells, Xenopus oocytes, and Chinese hamster ovary cells expressing the α-subunit of the rat type II sodium channel. In contrast to approved sodium channel-blocking antiepileptic drugs (AEDs) like carbamazepine, lamotrigine, and phenytoin, LCM did not appear to affect the fast inactivation of sodium channels but selectively enhanced slow inactivation. LCM shifted the slow inactivation voltage curve in the hyperpolarizing direction and significantly promoted the entry of channels into the slow inactivated state without altering the rate of recovery or altering the VGSC activation kinetics. The S-enantiomer (SPM 6953) and the major metabolite had no significant effect on sodium currents. Certain opiate analgesics, carbamazepine, and a phenytoin analog (Lenkowski et al., Neuropharmacology 52:1044-54,2007) have been shown to promote slow inactivation of VGSCs in dorsal root ganglion cells or central nervous system neurons, but they apparently also concurrently interact with fast inactivation (in contrast to LCM). Slow inactivation is thought to contribute to slow spike-frequency adaptation and to the termination of action potential bursts occurring as a result of prolonged or repetitive neuronal depolarization, and while the molecular rearrangement of the sodium channel that leads to slow inactivation is unknown, it is thought to be distinct from that of fast inactivation (Rogawski and Löscher, Nat Rev Neurosci 5:553-564,2004). The selectivity for slow inactivation may be reflected in the profile LCM displayed in animal seizure models where it differed in some respects from approved AEDs. For example, the classic sodium channel-modulating anticonvulsants are relatively inactive in the 6-Hz psychomotor model of treatment-resistant seizures, whereas LCM demonstrated efficacy in this test.

LCM did not show significant affinity for a range of common drug binding sites. However, using LCM analogs for affinity labeling, CRMP-2 was identified as a putative binding target. In a radioligand binding study with CRMP-2 expressed in Xenopus oocytes, binding of LCM to CRMP-2 was shown with an affinity of about 5 µmol/L. CRMP-2 is one of the five members of the CRMP-family of proteins (also called dihydropyrimidinase-related protein), implicated in developmental processes of the nervous system. Most of the five CRMP subtypes are highly expressed during early development, mainly in the CNS. CRMP-2 has been shown to be involved in neuronal differentiation, polarization and axonal outgrowth induced by neurotrophic factors such as brain derived neurotrophic factor (BDNF) or neurotrophin-3 (NT-3)(see **Fig. V.1.** below). Since CRMP-2 is thought to be involved in neuronal differentiation and control of axon outgrowth, the sponsor has speculated that this could produce disease-modifying effects, but there is no evidence for this other than an effect on kindling development in rats (discussed below). LCM was shown to interfere with these effects of CRMP-2, inhibiting neurotrophin-induced axonal outgrowth in an in vitro functional assay. Given the apparently crucial role of CRMPs in CNS development, the neurotoxic effects observed in the juvenile rat study (brain wts, behavioral deficits) are of particular concern. The failure to see anything in the pre- and postnatal study may have to do with

the different exposure periods, low exposures due to dose-limiting toxicity and indirect (ie, lactational) dosing, and/or the insensitive behavioral tests used.

LCM is structurally related to the endogenous amino acid D-serine, which acts as an NMDA receptor antagonist. An initial experiment had shown some affinity for the glycine binding site of the NMDA receptor (IC50: 5.2 µmol/L), although a more thorough study using specific ligands for a variety of NMDA receptor binding sites showed no significant (>50%) binding at a LCM concentration of 10 µmol/L. In another study using recombinant NMDA receptors expressed in Xenopus oocytes, LCM reduced NMDA and glycine induced currents in NR1-2B receptors (IC50=1.89 mM) independent of glycine concentration indicating that it could be acting as a noncompetitive NMDA antagonism at higher concentrations. Since this is a more than 30-fold the maximal therapeutic plasma concentrations, the sponsor thought it was unlikely to play a role in its mechanism of action or toxicity, but in vitro concentrations cannot be directly compared to plasma drug levels. LCM did not produce neuronal vacuolization or cell death in 2 rat studies (one conducted by [redacted] at single ip doses of up to 50 mg/kg. b(4)

LCM affected action potential duration in cardiac tissue in vitro and cardiac sodium currents in isolated cells, at concentrations which are achieved at the clinical MRHD, ie, 50 to 60 µmol/L (although in vitro and in vivo concentrations cannot be directly compared). Effects on sodium current were dependent on membrane potential, with higher inhibition at more depolarized potentials, suggesting the possibility of relevant effects under such conditions (eg, myocardial ischemia). Several mutations of sodium channels have been described which affect slow inactivation. These are associated with cardiac diseases such as Brugada syndrome, long QT interval, and AV block. However, the sponsor pointed out that none of these mutations appears to selectively affect slow inactivation, and they may also affect fast inactivation in a different manner. Based on these findings the sponsor conceded that enhanced slow inactivation may contribute to preexisting cardiac conduction disease and Brugada-like cardiac dysfunction. Dose-dependent cardiodepressant activity, prolongation of PR and QRS interval, and AV and ventricular block were seen in the safety pharmacology studies at plasma concentrations corresponding to those seen clinically at the MRHD, and, as expected, dose-related increases in PR interval and AV block have been observed in human trials of LCM.

In anticonvulsant screening tests, LCM blocked sound-induced seizures in mice with an ED50 of 0.63 mg/kg, ip and protected mice (ED50 = 4.5 mg/kg, ip) and rats (ED50 = 3.9 mg/kg, po) against maximal electroshock (MES)-induced tonic-extension seizures, but was ineffective in the threshold pentylenetetrazol (PTZ) test (**Table IIC.1**). This is the profile of several clinically used anticonvulsants (eg, phenytoin, carbamazepine and lamotrigine) which are thought to act by stabilizing the fast inactivated conformation of VGSCs by binding to sites on the sixth transmembrane segments in domains III and IV. In addition, LCM showed efficacy in the 6-Hz (low frequency, corneal electrodes) model for psychomotor seizures, with an ED50 of 9.99 mg/kg ip. LCM was active in the rat hippocampal kindling model (ED50 13.5 mg/kg), which is thought to predict activity against complex partial seizures, and also significantly inhibited kindling development in this model, which is speculated to indicate a potential effect on epileptogenesis, possibly related to modulation of CRMP-2. Daily administration of LCM during kindling acquisition produced a dose-dependent effect on kindling development (Brandt et al, Epilepsia 47: 1803–1809, 2006). Significant retardation of kindling was observed at 10 mg/kg/day, at which the average number of stimulations to reach kindling criterion was increased by >90%. However, while a significant inhibitory effect on kindling acquisition was also observed with 30 mg/kg/day, drug-induced seizures were observed in 50% of the rats treated this dose. These generalized seizures first occurred after 8 days of kindling, i.e., in partially kindled rats. Kindling is known to increase the sensitivity of rats to proconvulsant drug effects. Thus, the fact that seizures were observed after administration of 30 mg/kg LCM, but not lower doses of the drug, indicates dose-dependent proconvulsant activity in this model. The ability to retard kindling-induced epileptogenesis is shared with clinically established AEDs such as valproate, phenobarbital, lamotrigine, topiramate, or levetiracetam, but different from carbamazepine and phenytoin, which

do not retard kindling. However, in contrast to levetiracetam, the effect of lacosamide on kindling was lost after a washout period, which may suggest that it masked the expression of kindled seizures through an anticonvulsant action rather than exerting a true antiepileptogenic or disease-modifying effect. LCM was also active in a model of status epilepticus, blocking limbic seizures induced by self sustaining status epilepticus (SSSE) in rats. LCM did not block bicuculline- or picrotoxin-induced seizures. Although inactive against sc PTZ-induced threshold clonic convulsions, LCM elevated the seizure threshold somewhat in the iv PTZ test at the MES ED50; however, increasing the dose to that producing minimal motor impairment did not further elevate the threshold, again indicating the proconvulsant potential later shown in the toxicity studies.

Pharmacokinetics

LCM was rapidly and well absorbed (≥80%) after oral administration in all species. Kinetics were approximately linear in rabbits and dogs but less than dose proportionate in rodents. In mice, rats and dogs plasma exposure was similar between sexes. Although the half-life was short (t1/2 ≈1.3 and 1.8 hr reported in rat and dog PK studies, but t1/2 of 5 hr determined in rat carcinogenicity study TK report), accumulation was generally observed with repeated dosing in toxicity studies, presumably due to saturation of clearance at higher doses. In vitro protein binding was very low (unbound fraction 94, 95, 83 and 94% in mouse, rat, dog, and human, respectively). Following oral administration of labeled drug, radioactivity was rapidly and extensively distributed, with no evidence of specifically binding to any tissue. No significant melanin binding was observed in pigmented rats. [14C]-LCM-derived radioactivity crossed the placenta and was excreted in the milk. Following oral administration to rats for up to 7 days, there were no notable effects on the concentrations of hepatic microsomal protein and cytochrome P450 or on the activities of CYP1A and CYP2B. No major differences in systemic exposure to the major human O-desmethyl metabolite, SPM 12809, were observed between species, doses, or sex. The relative exposure to SPM 12809 in animals ranged from 22 to 51% of parent compound in terms of Cmax and from 25 to 73% in terms of AUC. In human plasma the relative exposure to SPM 12809 was less than 20% in terms of both Cmax and AUC at steady state. SPM 12809 is the only major systemically available human metabolite; it has weak pharmacological activity. Stereospecific analysis showed that there is no enantiomeric interconversion of LCM. There were no apparent route-related differences in LCM disposition following iv and oral administration of labeled drug to rats and dogs. In humans, LCM has an elimination half-life of approximately 13 hours, and a low metabolic turnover was observed. In the human ADME trial SP619, LCM (approximately 70% of the total radioactivity in the plasma sample), the main metabolite SPM 12809 (approximately 2% of the total radioactivity in the plasma sample), and traces of a polar fraction were detected in plasma after oral administration of 100mg [14C]-LCM in healthy subjects. After iv administration of 100mg [14C]-LCM, only LCM was detected in plasma. LCM PK parameters in the toxicity studies are compared to those in humans receiving the [redacted] 300 mg bid in **Table IIIA.1**, showing that there is little or no coverage in terms of AUC. It should be noted that human $AUC_{0\text{-}12h}$ values are used in the table, while $AUC_{0\text{-}24h}$ is given for animals. The human data are from a clinical trial in which multiple oral doses of 300 mg BID were administered to healthy subjects. Combining the two $AUC_{0\text{-}12h,ss}$ values would give an $AUC_{0\text{-}24h}$ of 245 ug.h/ml, which is similar to the $AUC_{0\text{-}\infty}$ value of 231 ug.h/ml reported after a single dose of 600 mg. The sponsor has argued that Cmax is more relevant than AUC, but while true for acute neurotoxic effects such as convulsions, which were dose limiting, it may not be true for other toxic effects. However, the Division is considering limiting the MRD to 400 mg based on the clinical dose-response data, which would improve the ratios somewhat. The $AUC_{0\text{-}24h,ss}$ for 200 mg bid is approximately 200 ug.h/ml (Veneeta Tandon, OCP). The iv and oral dosage forms are considered bioequivalent, and the same doses are to be used clinically by either route.

Toxicology

LCM has been evaluated for general toxicity in mice for up to 3 months, in rats for up to 6 months, and in dogs for up to 1 year. Reproductive and developmental toxicity studies were conducted in

rats and rabbits, juvenile studies in rats and dogs (preliminary study only), and 2-year carcinogenicity studies in mice and rats (**Table V.1**). The oral route was used for all pivotal studies. Genetic toxicity evaluations consisted of Ames test, mouse lymphoma assay, mouse micronucleus test, and rat UDS test. 2-week iv studies were performed in rats and dogs in support of an iv solution for acute use when oral administration is not feasible.

Table V.1. Toxicology studies of lacosamide

Study type and duration	Route of administration	Species
Single-dose toxicity		
(non-GLP)	oral, gavage	mouse, rat
	iv, bolus	mouse, rat
	oral, gavage	dog
Repeat-dose toxicity		
14 days	oral, gavage	mouse
13 weeks	oral, gavage	mouse
7 days (non-GLP)	oral, gavage	rat
28 days	oral, gavage	rat
30 days (non-GLP)	oral, gavage	rat
13 weeks	oral, gavage	rat
6 months	oral, gavage	rat
2 weeks	iv, bolus	rat
rising dose tolerance	oral, capsule	dog
2 weeks	oral, capsule	dog
30 days	oral, capsule	dog
3 months	oral, capsule	dog
12 months	oral, capsule	dog
rising dose tolerance	iv, bolus	dog
14 days	iv, bolus	dog
Genotoxicity	in vitro	Bacteria
		mammalian cells
	ip	mouse
	oral, gavage	rat
2-year carcinogenicity	oral, gavage	Mouse
		Rat
Reproductive and developmental toxicity		
fertility and embryo-fetal development	oral, gavage	rat
	oral, gavage	rabbit
embryo-fetal development		
pre- and postnatal development	oral, gavage	rat
juvenile animals	oral, gavage	rat
	oral, capsule	dog

In toxicity studies in all 3 species, neurological clinical signs were dose limiting. These included ataxia, abdominal and/or lateral position, reduced motility, tremor, and, at the highest doses, convulsions. These could be attributed to the pharmacological action of the drug.

Little in the way of chronic toxicity was observed. There was some evidence of liver toxicity, with increased alkaline phosphatase, cholesterol, triglycerides, and ALT (up to 90% in 2-year rat study) as well as increased liver weight and hepatocellular hypertrophy, but the changes were shown to be reversible and no more serious histological changes were observed. No evidence of liver toxicity was seen in mice and dogs. A diuretic effect was observed in some rat and dog studies. In dogs, the effect on blood pressure seen in the safety pharmacology studies was also observed in the 1 year toxicity study, but there were no clear effects on ECG and no heart

pathology was seen. However, as was pointed out by the sponsor, the exposures were lower than those shown to produce effects on cardiac conduction in the safety pharmacology studies and ECG measurements were not taken at the Tmax.

Findings in the 2-week iv studies were comparable to those seen after oral administration (signs of neurotoxicity including convulsions in dogs, increased transaminases in rats, diuresis). Plasma LCM levels at the high doses in these studies (extrapolated in rats) were similar to or greater than those in oral studies. In the 2-week dog study, 2nd degree AV block was observed in a single female at the highest dose tested (16 mg/kg). This is consistent with the effects on cardiac conduction seen in the safety pharmacology studies in dogs and monkeys as well as in clinical trials.

In genetic toxicity tests, LCM was positive in the mouse lymphoma assay but negative in the Ames test, mouse micronucleus test, and rat UDS test. The sponsor considered the positive results of the mouse lymphoma assay (**Table V.2**) to be a weak signal. They argued that concentrations above 2000 ug/ml (8 mM) were above appropriate testing levels, since according to ICH S2A the maximum treatment level for a freely soluble, nontoxic compound is 5 mg/mL or 10 mM (whichever is lower) for mammalian cells. They also cited the latest (2006) International Workshop on Genotoxicity Testing (IWGT; Moore et al, 2006), which states that a positive response only occurs when the increase in mutant frequency exceeds the Global Evaluation Factor (GEF) of 126. In this case, concentrations up to 8 mM were all below the GEF for both cultures, while at 12 mM or greater only one of the cultures was above this threshold. In the absence of S9, all mutant frequencies were below the GEF value and thus would be considered negative if this criterion were used. While the overall test results are clearly positive, with and without S9, the response is relatively weak, and in the absence of other evidence of genotoxic or carcinogenic potential, should not be a cause for concern.

Table V.2. Mouse lymphoma assay – summary of induced mutant frequencies

Lacosamide Test Concentration		**Duplicate**	**Induced Mutant Frequency (IMF)**		
			Assay #1		**Assay #2**
µg/mL	**mM**		**-S9 (4h)**	**+S9 (4h)**	**-S9 (24h)**
250	1	A	n.t.	n.t.	-1
		B	n.t.	n.t.	8
500	2	A	13	n.t.	2
		B	-4	n.t.	-6
1000	4	A	12	27	-4
		B	6	33	-16
2000	8	A	49	81	6
		B	64	110	23
3000	12	A	87	134	76
		B	85	116	63
4000	16	A	T	124	n.t.
		B	T	181	n.t.
5000	20	A	n.t.	119	n.t.
		B	n.t.	167	n.t

n.t.: not tested; T: too toxic to count (total growth <10%)

The 2-year studies did not indicate any carcinogenic potential for LCM. In mice (CD-1; oral [gavage] doses of 20, 60 or 180 mg/kg for 104 weeks), dose selection was appropriate based on clinical signs. The HD was associated with ataxia, reduced motility, abdominal position, tremor, and clonic convulsions. There were no treatment-related effects on survival, but BW was decreased in HD males (10% below C at termination). There were no clinical pathology findings and no apparent treatment-related effects on incidences of neoplastic or non-neoplastic lesions. An $AUC_{0\text{-}24h}$ of approximately 230 µg·h/mL was reached at the HD (no sex difference) at the end of the study.

In rats (CD; oral [gavage] dose of 40, 80 and 160 mg/kg in males and 40, 80 and 160/180/200 mg/kg in females for 104 weeks), dose selection was based on clinical signs. Abdominal position, reduced motility, and clonic convulsions were seen in HD males and females. No treatment-related change in survival was noted, but a decrease in BW gain was seen in HD males (BW 8% below C at termination). Increases of the absolute and relative liver weights and ALT (up to 90%) were seen in MD and HD males and females, but the histopathological examination indicated no treatment-related differences in incidences of neoplastic or non-neoplastic lesions. $AUC_{0\text{-}24h}$ values of 605 and 737 µg·h/mL were reached in males and females, respectively, at the HD at the end of the study.

Reproductive and developmental toxicity studies showed some evidence of developmental toxicity but no teratogenic potential; however, exposure margins at the highest doses tested were minimal compared to exposures expected clinically. In a combined fertility and embryofetal development study in rats (oral [gavage] doses of 20, 75, or 200 mg/kg; administration prior to and during mating and through organogenesis), clinical signs of toxicity and reductions in parental body weight gain were seen at the HD, but there were no apparent effects on mating and fertility or on C-sectioning and litter parameters. Although maternal toxicity was less than expected during the gestational treatment period in females, presumably due to tolerance (pharmacodynamic/physiological) development during the premating period, the dose range-finding study indicated that dose selection was appropriate for the gestational period and rat TK data show increases or no change in plasma drug levels over time; therefore, the combined study design and assessment of effects on fertility and embryofetal development could be considered acceptable, although the negative results should not necessarily be considered reassuring because of the uncertainty about relative exposures. The sponsor was told at the EOP2 meeting that a stand-alone embryofetal development study would be preferable, particularly for this class of drug, and this should still be considered, with bid dosing to increase exposure and better mimic the clinical exposure pattern.

Doses were based on an embryofetal range-finding study (100, 200, or 300 mg/kg on GD 7-17), in which maternal BW gain was significantly reduced (20 and 60% compared to C over GD 7-20) and resorptions were increased (1.0, 0.7, 1.6, 2.2 % resorbed/litter in C, LD, MD, and HD) at the MD and HD, and fetal BWs were reduced at the HD (30% compared to C). No fetal gross alterations were reported. TK data from this study (**IVE.1.1**) indicates that maximum exposures at the HD used in the definitive study were less than those expected clinically and lower than those measured in nonpregnant rats. However, AUCs were underestimated due to the short time interval used (0-4 hrs). PK values for the LD and HD were comparable to those measured in the 13 week rat toxicity study when AUCs from that study were reanalyzed using the same time interval, ie, 0-4 hrs, indicating that there may not be a significant effect of pregnancy. $AUC_{0\text{-}8}$ values in the 13-week study were about 150 and 200 ug.h/ml at 100 and 300 mg/kg, respectively. These values are still underestimates, since high plasma levels were present at the 8 hr time point in this study. Based on levels measured at the beginning of the 6 month study ($AUC_{0\text{-}24h}$ of 400 and 480 ug.h/ml in males and females at the HD of 180 mg/kg), maximum exposures evaluated in the definitive embryofetal study would probably have been somewhat (at least 2-fold) higher than those expected clinically (200 and 250 ug.h/ml at 400 and 600 mg/day). And there is also a small safety margin for Cmax (also about 2X), which has been shown to be more important for some teratogenic drug effects.

In the rabbit embryofetal development study (oral [gavage] doses of 6.25, 12.5, or 25 mg/kg on GDs 6 -18), there were no apparent adverse effects on development. The dose range-finding study indicated that maternal convulsions were dose-limiting, so the study can be considered adequate for assessment of effects on embryofetal development; however, maximum exposures ($AUC_{0\text{-}24h}$) were similar to or lower than those expected clinically at [redacted] either 600 or 400 mg/day). Doses were based on an embryofetal range-finding study (6.25, 12.5, 25, or 50 mg/kg) in which maternal convulsions and BW reduction occurred, resorptions were increased (0, 0, 2.9, 2.9 % resorbed/litter) at the MD and HD and fetal body weights were reduced at the HD (12% compared to C). Although no fetal gross alterations were reported, the finding of increased embryofetal death at doses at or just above the maternal MTD agrees with what was seen in rats. It is not clear, however, why the increased resorption seen in the dose range-finding studies, at 200 mg/kg in rats and 25 mg/kg in rabbits, was not replicated in the definitive studies.

b(4)

In the pre- and postnatal development study in rats (oral [gavage] doses of 25, 70, or 200 mg/kg from GD 7 through PND 20), the mean duration of gestation was significantly prolonged in all treatment groups and developmental toxicity was seen in increased numbers of stillborn pups at all doses (SS at LD and HD), and increased neonatal pup mortality, decreased numbers of liveborn pups at HD, and decreased offspring BWs. As is often the case, it is not clear whether the prolonged gestation was a cause or result of the stillbirth seen in LCM-treated dams. These two endpoints are often correlated. It was noted that clinical signs became more severe near term, and there were 2 HD deaths on GD23, associated with what appeared to be dystocia. However, prolonged gestation and increased stillbirth were also seen at the LD and MD which were not associated with clinical signs, although the effects were small, not dose-related, and did not result in reduced live litter size. In addition, neonatal deaths occurred at the HD after maternal clinical signs had subsided. There was some suggestion of an effect on offspring learning and memory as assessed in passive avoidance and M-maze performance, although none of the differences reached statistical significance. There were no apparent developmental effects on other endpoints evaluated, including landmarks of sexual maturation and reproductive performance. Based on the maternal toxicity observed (clinical signs and BW gain reduction), dose selection for this study was appropriate. The failure to see clear behavioral or other evidence of developmental neurotoxicity should not allay concerns, since there is no safety margin at the high dose based on plasma exposure and the tests conducted are relatively insensitive.

In a juvenile rat study (oral [gavage] doses of 30, 90, or 180 mg/kg for 6 weeks beginning on PND 7), BW gain was decreased during lactation, sexual maturation was delayed in females, absolute and relative brain weights were decreased, and neurobehavioral changes (altered open field performance, deficits in Morris Maze learning and memory) were observed (**Tables IVE.3.4-8**), primarily at the HD (altered open field performance also seen at MD), which was associated with exposures ($AUC_{0\text{-}8h}$) to parent ranging from about 1000 ug.h/ml at the start of dosing to about 300 ug.h/ml at the end of the dosing period. Plasma exposure was initially considerably higher in juvenile rats compared to adult rats receiving the same doses, but by the end of the study levels were similar to those seen in adults. The brain weight effects were considered secondary to general delayed development by the sponsor; however, the brain is spared to varying extents in nutritional deprivation-induced growth deficit, and relative brain wts were also D-D decreased in females at the early time point. The brain weight and behavioral changes seen in juvenile rats, and possible subtle behavioral effects in the pre- and postnatal study (where brain weights were not measured), indicate a potential for developmental neurotoxicity that may warrant further study. Because pharmacological investigations indicated an interaction between LCM and CRMP-2, a protein thought to be involved in nervous system development, these findings are clearly not unexpected. In functional studies LCM was shown to inhibit the CRMP-2-mediated effects of neurotrophins (NTs) on axonal outgrowth of primary hippocampal cells, at concentrations as low as 1 μM (**Fig. V.1** below). One question for possible follow-up studies is whether the critical period is primarily late in development, as indicated by the juvenile study, or would extend to earlier periods if higher doses or more sensitive measures had been used in the

pre- and postnatal study. In any case, the vulnerable period for LCM-induced developmental neurotoxicity may need to be better defined.

The relevance of the juvenile study results to human pregnancy exposure is also an important question. Efforts to correlate the timing of human and rodent brain development (e.g., Dobbing and Sands, Early Hum Dev 3:79-83,1979; Broening et al., J Neurosci 21:3228-35,2001; Avishai-Eliner et al., Trends Neurosci 25:518-24,2002) indicate that the majority of structural and functional milestones occurring during the first week of life in the rat hippocampus take place during the third-trimester gestational period of the human. CNS development at birth in humans has been roughly equated to that in the rat on postnatal day 10. Therefore, some of the exposure period in the juvenile rat study may correspond to exposure during the third trimester of human pregnancy. Therefore, the juvenile rat data should probably be included in the pregnancy section of labeling, along with some discussion of the potential for developmental neurotoxicity based on the interaction of LCM with CRMP-2.

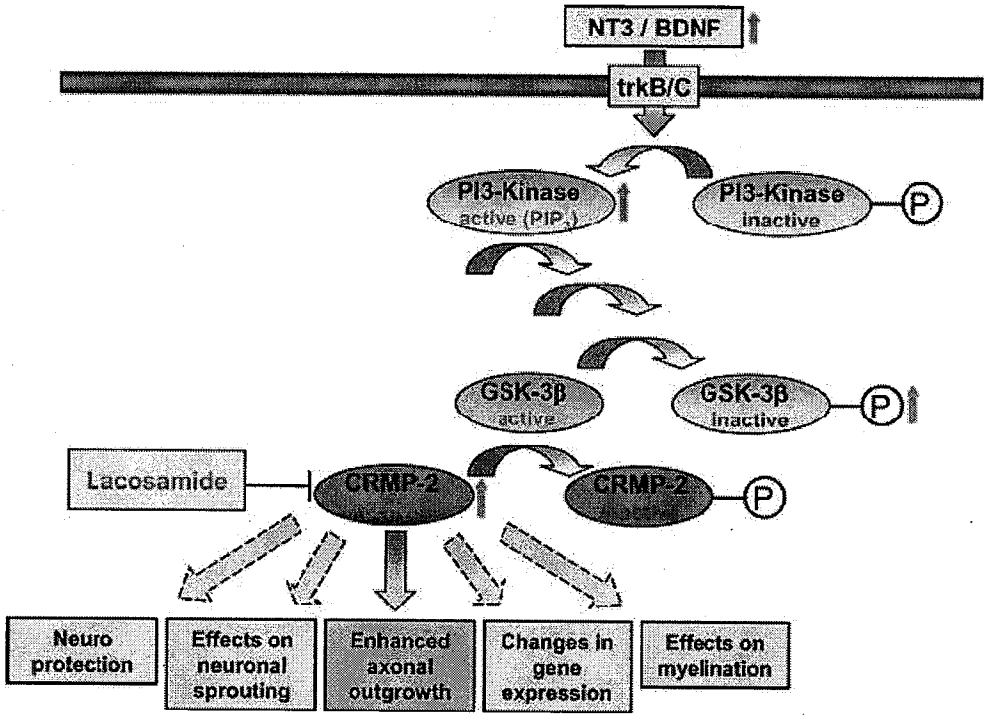

Fig. V.1. Schema showing CRMP-2-mediated transduction of neurotrophic signals to neuronal response and the possible interaction of lacosamide. Neurotrophins like NT-3 and BDNF activate their receptors in the plasma membrane, triggering a transduction cascade, which regulates the activity of intracellular protein kinases (e.g., PI3-kinase or GSK-3β) finally resulting in increased levels of active CRMP-2. Active, nonphosphorylated CRMP-2 has been shown to enhance axonal outgrowth and might also be involved in the induction of other cellular responses. Interaction site of lacosamide is indicated. (Taken directly from Beyreuther et al, CNS Drug Reviews,. 13:21-42,2007)

During rat development mRNA for CRMPs is expressed during late embryonic life (present by at least GD13) and decreases in abundance shortly after birth, with adult levels of CRMP-2 being ~15% of PND 1 levels (Wang and Strittmatter, J Neurosci 16:6197–6207, 1996). The expression of the four CRMP mRNAs displays distinct temporal and spatial regulation. CRMP-2 exhibits the most ubiquitous neuronal expression pattern, being present early in development in a majority of neurons and in a selected group of adult neurons, such as pyramidal cells of the hippocampus,

Purkinje cells of the cerebellum, and sensory neurons of dorsal root ganglia. The persistent expression in adult hippocampus and cerebellum suggests that CRMPs may function in the adult brain as well as during brain development. Both the hippocampus and the cerebellum have been identified as areas with high degrees of synaptic remodeling associated with learning, memory, and plasticity. CRMP-1 knock-out (CRMP-$1^{-/-}$) mice reportedly show a reduction in long-term potentiation (LTP) in the CA1 region and impaired performance in hippocampal-dependent spatial learning and memory tests as adults (Su et al, J Neurosci 27:2513-24, 2007). Adult *CRMP-$1^{-/-}$* mice exhibited intense microtubule-associated protein 2 (MAP2) staining in the proximal portion of the dendrites, but reduced and disorganized MAP2 staining in the distal dendrites of hippocampal CA1 pyramidal cells. Immunoreactivity to GAP-43 (growth-associated protein-43) and PSD95 (postsynaptic density-95, a postsynaptic membrane adherent cytoskeletal protein) was also decreased in the CA1 region of the knock-out mice. Although the expanded CNS histopathology examination of juvenile rats did not reveal any obvious pathology using standard H&E, more sophisticated techniques, eg, immunohistochemical examination using neuronal structure specific markers, may be necessary for a proper assessment of possible LCM effects on brain development, both pre- and postnatally. The selectivity of LCM for CRMP-2 vs other CRMPs is not known.

Clinical and nonclinical studies have repeatedly suggested that AED use during pregnancy may produce cognitive impairment in the offspring (Fisher and Vorhees, Pharmacol Res 26:207-21, 1992; Meador et al., Epilepsy and Behavior 11: 292-302, 2007). A recent literature report (Manent et al, Epilepsy Research 78:131-139, 2008) found that prenatal exposure of rats to lamotrigine (LTG) during the critical period for neocortical and hippocampal neuronal migration (GDs 14-19) induced hippocampal and cortical malformations at maternal plasma concentrations within the therapeutic range. LTG is considered to act primarily by reducing the release of glutamate through inhibition of voltage-dependent sodium channels, although a variety of effects on other ion channels and transmitters have also been reported (Leach et al. In: Antiepileptic Drugs (2002), pp. 364–369). Neuronal migration is known to be modulated by neuronal activity, neurotransmitters such as GABA and glutamate, and a variety of voltage-gated ion channels (Manent and Represa, Neuroscientist **13**:268–279, 2007). So the potential for LCM-induced developmental neurotoxicity via multiple mechanisms may exist.

One interesting aspect of the juvenile rat study was that much higher exposures to parent were seen than in adults at the same dose, and that these were well tolerated, ie, not associated with the convulsions seen in adults. TK showed that levels of parent decreased over the course of treatment (**Table IVE.4.1**), with 2-4-fold higher Cmax and 3-5-fold higher AUC values on treatment day 1 compared to treatment day 42, respectively. Compared to the 13-week rat toxicity study in adults, Cmax and AUC on treatment day 42 were generally comparable, except for AUCs at the MD and HD, which were still about 2-fold higher in the juvenile animals. The opposite trend was found for the O-desmethyl metabolite (SPM 12809), for which Cmax and AUC increased over the course of treatment of juvenile animals. The relative exposure to SPM 12809 was less than 10% after the first dose, and ranged from 18 to 61% by the end of the dosing period, ie, levels comparable to those seen adult rats. This could implicate the metabolite in the convulsions seen in adults, or reflect age-dependent seizure susceptibility, as has been shown with other convulsant drugs (Cavalheiro et al, Brain Res, 465:43-58,1987).

Impurities

The original NDA specified levels of 2 impurities that were above the qualification threshold. A specification limit of NMT [redacted] was proposed for drug substance impurity [redacted]), which is above the ICH limit of 0.15%. This impurity was adequately tested in the chronic oral toxicology (6-month rat, 12-month dog), reproductive toxicology, and genetic toxicology studies for LCM. However, [redacted] was not detectable in the drug batch used in the rodent carcinogenicity studies. Although carcinogenicity testing of impurities is not generally required, there was concern regarding the genotoxic potential of [redacted] because of the

b(4)

positive results obtained in the in vitro mouse lymphoma tk assays. Therefore, the sponsor was told that they would need to either lower the drug substance specification or conduct genetic toxicity testing of [redacted] directly in order to support the proposed specification limit. b(4)

For the iv formulation [redacted] drug product, a specification limit of NMT [redacted] was proposed for a degradant, [redacted] which is well above the ICH limit of 0.20%. The presence of [redacted] in mice provided the basis for qualification of the impurity at this level [redacted] n the mouse in which a similar toxicity profile was seen with LCM compared to other species); however, it was pointed out that the toxicological evaluation of LCM in mice did not include an assessment of effects on embryo-fetal developmental, which we generally require for qualifying impurities. The sponsor was told that without information on the potential developmental toxicity of [redacted] we could not approve an acceptance criterion greater than 0.20%. b(4)

The sponsor responded by agreeing to tighten the limit for [redacted] to NMT [redacted] thus obviating the need for toxicological qualification. They further contended that the signal in the mouse lymphoma assay was weak (see gentox discussion above), and pointed out that data from other assays in which the same drug substance batch or a batch with an even higher content of [redacted] were used did not indicate genotoxic activity. The sponsor also provided a PK study that had been performed since the initial NDA submission which showed that [redacted] The relative systemic exposure to SPM 6912 in relation to LCM was [redacted]. Extrapolating from this data, it was determined that in the rabbit embryofetal studies animals would have been exposed to levels of [redacted] similar to those expected in clinical use. This information, in combination with the mouse data, was considered adequate to qualify the impurity at the proposed level. b(4)

VI. RECOMMENDATIONS

The applications (oral and iv) can be approved from a pharmacology/toxicology standpoint. Labeling recommendations are given below. The sponsor should address the following in Phase 4:

1) The mechanism of action and juvenile rat studies of lacosamide indicated a potential for developmental neurotoxicity which needs to be more fully characterized. The sponsor should examine the effects of lacosamide on brain development during the prenatal and early postnatal periods using more sensitive techniques for assessing CNS structure and function than were employed in the standard pre- and postnatal development study. They should also explore the use of multiple daily dosing as a means of achieving higher plasma drug exposures during pregnancy and better mimicking the human exposure pattern.

2) Although the rat and the rabbit embryofetal development studies of lacosamide were adequate under the dosing conditions usually employed (ie, qd), the maximum plasma drug exposures tested were relatively low (or uncertain) compared to those expected clinically. Therefore, the sponsor should address the potential for increasing exposure with divided dosing in a study of this type.

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_____ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

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PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: —, 22-253, 22-254, — b(4)

SERIAL NUMBER: **N 000**

DATE RECEIVED BY CENTER: **9/28/07**

PRODUCT: **Vimpat™ (lacosamide)**

INTENDED CLINICAL POPULATION: **Management of neuropathic pain associated with diabetic peripheral neuropathy and Adjunctive Therapy Treatment of Partial Onset Seizures in Patients with Epilepsy**

SPONSOR: **Schwarz Bioscience, Inc.**

DOCUMENTS REVIEWED: **Original Electronic Submission**

REVIEW DIVISION: **Division of Anesthesia, Analgesia, and Rheumatology Products (HFD-170) and Division of Neurology Products (HFD-120)**

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Specific NDA details:

NDAs	Product	Indication
22-253	Lacosamide Tablets	Adjunctive Therapy Treatment of Partial Onset Seizures in Patients with Epilepsy
22-254	Lacosamide Injection	
—	—	
—	Lacosamide Tablets	Management of Neuropathic Pain Associated with Diabetic Peripheral Neuropathy

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability
From the nonclinical pharmacology and toxicology perspective, based upon the information reviewed by this reviewer, NDA —— may be approved.

B. Recommendation for nonclinical studies
No additional nonclinical studies are required, based upon the materials reviewed by this reviewer. The reader is referred to the review by Dr. Edward Fisher for recommendations regarding additional reproductive and developmental toxicology studies.

C. Recommendations on labeling

See the review from Dr. Edward Fisher for NDA 22-253 for recommendations on labeling.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

In support of NDA —— , the Sponsor has completed the appropriate preclinical studies including toxicology studies in rats, mice and dogs with duration of single dose to 12 months, 2-year carcinogenicity studies in mice and rats, standard battery of genotoxicity studies, and reproductive toxicity studies in rats and rabbits.

The major target organs of toxicity were brain/central nervous system, gastrointestinal tract, and cardiovascular system. The CNS toxicity was manifested by sedation, decreased locomotor activity, ataxia, tremors and convulsions. Cardiovascular toxicity was manifested as dose-dependent cardiodepressant activity, prolongation of PR and QRS interval, and AV and ventricular block. These cardiac effects were seen at plasma concentrations corresponding to those seen clinically at the MRHD.

B. Pharmacologic activity

Pharmacology:
Lacosamide, (R)-2-acetamido-N-benzyl-3-methoxypropionamide, is a member of a series of functional amino acids. Nonclinical experiments has suggested that lacosamide has a dual mode of action underlying its anticonvulsant and analgesic activity. In vitro electrophysiological studies have shown that lacosamide

selectively enhances slow inactivation of voltage-gated sodium channels, resulting in stabilization of hyperexcitable neuronal membranes and inhibition of repetitive neuronal firing while exerting no effects on physiological neuronal excitability. Also, it has been shown that lacosamide binds to collapsin response mediator protein-2 (CRMP-2), a phosphoprotein which is mainly expressed in the nervous system and is involved in neuronal differentiation and control of axonal outgrowth.

Pharmacodynamics: b(4)

Pharmacodynamic characterization of lacosamide was conducted in several animal models of experimental pain. Results from these animal studies demonstrated that ...

Safety Pharmacology:

Nonclinical safety pharmacology studies included neurological, cardiovascular, pulmonary, renal and gastrointestinal (GI) safety studies. Results from these safety pharmacology studies did identify effects on the central nervous system, cardiovascular system, renal and gastrointestinal system. The effects of lacosamide on several behavioral parameters were assessed in neurological screening tests conducted in mice and rats and the Irwin test conducted in rats. The neurological effects of lacosamide were qualitatively similar in both rat and mice; lacosamide produced dose-dependent CNS depressant effects. Lacosamide-induced depressant effects included sedation, reduced spontaneous locomotor activity, impairments of motor coordination, decreased body and abdominal muscle tone, ataxia and tremors. Evaluation of orally administered lacosamide in rats in Irwin test also demonstrated that lacosamide produces CNS depressant effects in rats. Decreased muscle tone was observed at 32 mg/kg. Sedation, Straub tail and rolling gait were observed at a dose of 64 mg/kg. At 256 mg/kg, tremor, loss of balance, decreased respiration, loss of grasping, loss of fear, analgesia and marked hypothermia were observed. Lacosamide-induced depressant effects were noticeable at doses of 300 mg (bid) in humans.

Results from toxicology studies provided evidences that lacosamide can induce convulsions in rats, mice and dogs.

Cardiotoxicity could be a potential adverse effect of lacosamide. In vitro studies with isolated canine Purkinje fibers showed that lacosamide at concentrations of 15, 50 and 150 μmol/L, produced statistically significant reduction of action potential duration (APD 90 was decreased by 5%, 15% and 30% at 15, 50 and 150 μmol/L, respectively) and reduced (9% and 32% at 50 and 150 μmol/L, respectively, non-statistically) the maximal upstroke velocity (Vmax) in isolated canine Purkinje fibers. These effects were observed at concentrations that were 1.2 to 10 times the human exposure at — (600 mg/day based on Cmax of b(4)

14.5 ±1.7 μg/mL (58 μmol/L)). In vitro studies with recombinant human hERG channel showed that lacosamide minimally affected hERG current at concentrations as high as 300 μmol/L; it produced a potassium current block of 7%.

Lacosamide has potential cardiodepressant activity. In vivo studies in anesthetized dogs showed that lacosamide (2, 4, 8 and 12 mg/kg, cumulative application with a 30-min dosing interval) induced dose-dependent hypotensive effects. Hypotensive effects started at 2 mg/kg and 4 mg/kg in female and male dogs, respectively. At 12 mg/kg, a 13% reduction in mean arterial pressure was measured. Lacosamide-induced hypotensive effects were short lasting and appeared at the time of maximal drug plasma level which was associated with plasma levels of 11.3 to 22.6 μg/mL. These plasma levels were at — equivalent plasma concentration (Cmax of 14.5 ± 1.7 μg/mL). The hypotensive effects of lacosamide were not associated with effects on blood vessels because no effect on peripheral resistance was observed. Also, at all doses tested, a slight but statistically significant increase in heart rate was observed (3% to 7%). Consistent with the cardiodepressant action of lacosamide, a reduction in contractility was observed; systolic left ventricular pressure, dP/dt and cardiac output was decreased by 7-9%, 12-17% and 6-7%, respectively. In addition, the results suggested that lacosamide slows atrio-ventricular and ventricular conductivity as evidence by the lacosamide-induced increase in PR interval and QRS duration. Similar results were observed in another in vivo study in which anesthetized dogs (1M and 1F) were consecutively administered lacosamide at doses of 2.5, 5, 10 and 15 mg/kg with at least 30 minute between doses. Dose-related changes in BP, CO and LVP were observed in 10 and 15 mg/kg in the male. At 10 mg/kg, maximal reduction in MAP (14%), CO (22%) and LVP (14%) were observed. At 15 mg/kg, maximal reduction in MAP (51%), CO (64%), LVP (37%) and HR (24%) was observed. The marked decreases in MAP, CO and HR appeared at the same time as AV dissociation. Similar effects were observed in the female dog. She died at the conclusion of the end of the 15 mg/kg dose.

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Safety assessment of lacosmide effects on renal function in Long Evans rats did not demonstrate any lacosamide-induced effects on renal function. Lacosamide did not show an influence in urine volume or excretion of sodium and potassium ions within 6 hours after administration. However, results from several repeat toxicity studies in rats indicated that lacosamide can produce diuretic-like effects. In a 13-week repeat-toxicity study, orally administered lacosamide produced changes indicative of drug-induced diuretic effects. A significant increase in urine volume was observed in both genders. Also, lacosamide induced significant dose-dependent decreases in the following urine chemistry: chloride, urea nitrogen, creatine, sodium and potassium. A 6-month oral toxicity study conducted in rats also produced evidence of diuretic-like effects of lacosamide. In males, at a dose of 180 mg/kg/day, urine volume (not statistically significant) was increased by 24% and 12% during weeks 13 and 26, respectively. Compared

to the control, urine volume was increased in females in the mid-dose group by 43% and 46% during weeks 13 and 26, respectively. At 180 mg/kg, urine volume was increased in the females by 32% (week 13) and 87% (week 26). Also compared to the control group, the high dosed female water consumption was transiently increased up to 21% in week 6. Specific gravity was minimally, but statistically significantly decreased in females at 90 and 180 mg/kg/day. Diuretic-like effects of lacosamide was also observed in dogs in a 12-month oral toxicity study. Consistent with rat study results, female dogs appear to be more sensitive to lacosamide renal effects. Females administered lacosamide at a dose level of 10 mg/kg/day had a significant increase in urine volume (57%) compared to control.

Gastrointestinal tract is a potential target organ for lacosamide. Nonclinical data shows that lacosamide has the potential to cause constipation. In vivo assessment of lacosamide effects on gastrointestinal function in rats showed that lacosamide can delay gastrointestinal transit time. Lacosamide elicited a dose-dependent decrease in intestinal motility. Significant decrease in gastrointestinal transit time was evidence at the lowest dose tested (1.0 mg/kg) and reached a maximum at the highest dose tested (25 mg/kg). Compared to the vehicle control, GI motility was inhibited by 7%, 8%, 15%, 28%, 28%, and 27% following oral administration of 1, 3, 10, 25, 50 and 75 mg/kg, respectively. These findings suggest that lacosamide has the potential to induce constipation.

Pharmacokinetics:
Lacosamide is well absorbed following oral administration. Absolute bioavailability of lacosamide is high in rats (94%) at a 10 mg/kg dose. In dogs, bioavailability was greater than 70% at a 10 mg/kg dose. Urine is the principal route of ^{14}C excretion following ^{14}C-lacosamide administration. Excretion pattern was similar in mice, rats, dogs and man. The rate of excretion was rapid in all species with the majority of the dose recovered within 24 hours. In rat and dog, > 75% (mean) and 73% to 80% ^{14}C-lacosamide oral dose is present in the 0-24-hr urine sample. In the mouse, > 80% of the ^{14}C-lacosamide following oral administration was recovered within 48 hours.

Metabolism of lacosamide is extensive with 12.7%, 18.9%, 7.1% and ≈ 40% of unchanged parent compound being eliminated in the urine of mice, rats, dogs and humans respectively, following single oral administration of ^{14}C-lacosamide. In vitro metabolism studies using isolated hepatocytes from the liver of male mouse, rat, Beagle dog, human and female New Zeland White rabbit demonstrated a similar metabolite profile; three metabolites were detected. Each species produced only two major metabolites. SPM 12809, the O-desmethyl metabolite, was common to all species. The desacetyl metabolite, SPM 6912, was recovered in the suspension cultures of both human and mouse. Hydroxylation was favored in rat, rabbit and dog. Results from the in vitro study showed that the metabolism of ^{14}C-lacosamide in hepatocytes from human qualitatively resembled its metabolism in the mouse. In an in vitro rat model using liver microsomes, 4

significant metabolites were discerned: SPM 12809, SPM 12817 (p-hydroxy metabolite), traces of SPM 6912 and two unknown polar metabolites. In an in vitro human model, 3 significant metabolites were detected. SPM 12809 and SPM 6912 were detected in liver microsomes; and a polar metabolite was identified using recombinant CYP2C19 microsomes. The major pathway of metabolism involved O-demethylation of lacosamide to form SPM 12809. Other pathways involved deacetylation and hydroxylation.

^{14}C-lacosamide is widely distributed in most tissues and crosses the blood brain-barrier in rat, mice and dog. The highest concentrations of ^{14}C-lacosamide-derived radioactivity had the propensity to be associated with organs associated with biotransformation and excretion, such as the liver and kidney, and the lachrymal gland. Concentrations of ^{14}C-lacosamide-derived radioactivity were at their maximum at the earliest time points (0.5 and 1 hours).

Repeat-dose toxicology:

The nonclinical toxicology program to support the chronic indication consisted of repeat-dose toxicology studies of up to 13 weeks duration in mice, 6-months in rats and 12 months in dogs. Chronic treatment with lacosamide produced evidences of CNS toxicity in all species. In the mouse, ataxia and reduced motility were noted at a dose level of $\geq$ 120 mg/kg/day. Mice also exhibited abdominal position.

Oral administration of lacosamide to rats for 13-weeks and 6-months produced treatment-related clinical signs. Oral administration of lacosamide for 13-weeks to rats produced ataxia, hypoactivity and convulsions (female only) at a dose level of 300 mg/kg/day. Treatment-related mortalities were observed in females dosed with 300 mg/kg/day. Clinical chemistry changes that were notable included an increase in alkaline phosphatase, total cholesterol and alanine aminotransferase with dosing $\geq$ 100 mg/kg/day in females and/or males. Electron microscopy analysis of the liver from females in the high dose group revealed hypertrophy of hepatocytes with increases of mitochondrial and rough endoplasmic reticulum in the cytoplasm of the hepatocytes. In light of these observed changes, no degenerative changes in cellular organelles of the hepatocytes were noted. Therefore, one may conclude that these changes may represent a physiological adaptive change to the increase in alkaline phosphatase and alkaline phosphatase level measured in the females.

Repeat-dose toxicity study of 6-months duration in the rat revealed several treatment-related effects. At a dose level of 180 mg/kg/day, the primary clinical signs included excessive salivation, reduced motility and apathy. The onset of these clinical signs were rapid and persisted for hours; starting 15 to 20 minutes after dosing and lasting a few hours (reduced motility) or up to 24 hours (salivation). Consistent with the effects observed in the 13-week repeat-dose study, ALT was increased following dosing with 180 mg/kg/day. Relative to

control, ALT was significantly increased by 43% at week 13 in the females. Relative liver weight and liver-to-brain weight ratio were increased by 13.3% and 14.8%, respectively, in high dose females. The effects were reversible, liver weight was within normal range after a four week recovery period.

In the dog, tonic-clonic convulsions were the primary treatment-related toxicity associated with the 25 mg/kg/day of lacosamide. Other treatment-related overt signs of toxicity observed following 25 mg/kg/day were ataxia, reduced motility, tremor and increased salivation. Assessment of cardiovascular parameters indicated a reduction on peripheral arterial blood pressure in males in the 5 mg/kg/day group and females in the 10 and 20/25 mg/kg/day group.

The following table describes the exposure safety margins between the NOAEL in nonclinical studies and the expected therapeutic exposure to lacosamide at — dose of 600 mg/day. b(4)

		Exposure to Lacosamide at the Nonclinical NOAEL and the Predicted Human Exposure with — Use of Lacosamide (600 mg/day based on Cmax of 14.5 ± 1.7 µg/mL (58 µmol/L)) b(4)					
		NOAEL				Ratio	
Species	**Study Duration**	**(mg/kg/day)**	**(mg/m^2)**	**Cmax (µg/mL)**	**HED (mg/kg)**	**C_{max}**	**mg/m^2**
Mouse	**3-months**	**60**	**180**	**29.04 (males) 27.62 (females)**	**4.86**	**2**	**0.5**
Rat	**13-week**	**100**	**600**	**27.033 (males) 36.033 (females)**	**16.2**	**1.9 (males) 2.5 (female)**	**1.6**
Rat	**6-month**	**90**	**540**	**27.45**	**14.5**	**1.9**	**1.5**
Dog	**12-month**	**10**	**200**	**15.52 (males) 13.15 (females)**	**5.41**	**1.0 (male) 0.9 (female)**	**0.5**

As indicated in the table, the safety margin for all species was less than 10; thus indicating a small margin of exposure or safety for human use. The dog was the most sensitive specie to the lacosamide-induced CNS toxicity. The dog was the most sensitive to lacosamide-induced convulsions. Convulsions were observed following repeated oral administration at a dose level of 25 mg/kg/day for 12-months. The table below shows the safety margin to be in the range of 2.5 to 7.5 when it is based on dose (mg/kg). However at the — of 600 mg/day, the safety margin is 1.0 when based on the exposure level (C_{max}). b(4)

	Nonclinical Data			Human Dose (mg/day) Safety margin (Animal Dose/Human Dose)		
Species	Dose (mg/kg)	AUC (h.µg/mL)	Cmax (µg/mL)	200 (3.33 mg/kg)	400 (6.6 mg/kg)	600 (10 mg/kg)
Dog	25	133.2	21.37	7.5	3.8	2.5

C. Nonclinical safety issues relevant to clinical use

The major toxicity findings and their clinical relevance are listed below:

- The CNS-related clinical signs of sedation, decreased locomotor activity and ataxia noted in all species studied suggest similar treatment-related clinical signs are predicated to be observed in human.

- The decrease in gastrointestinal motility observed in rats suggests that lacosamide may cause constipation in humans.

- Cardiotoxicity is a concern of lacosamide. Cardiodepressant activity and ECG changes were observed in dogs. Prolongation of PR and QRS interval in ECG was also observed in dogs and was associated with first AV block.