REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

KEY WORDS: Kepra, levetiracetam, UCB Pharma Inc, epilepsy
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Sponsor: UCB Pharma Inc, Smyrna GA, USA

Manufacturer for drug substance – UCB SA Pharma Sector, Chemin du Foriest, Belgium

Drug:

Code Name: **ucb L059**, ucb 22059, SIB-S1
Trade Name: **levetiracetam, Kepra**
Chemical Name: (s)-alpha-ethyl-2-oxo-pyrrolidineacetamide
CAS Registry Number: 102767-28-2
Molecular Formula/ Molecular Weight: $C_8H_{14}N_2O_2$,170.21

Relevant INDs/NDAs/DMFs: [redacted]

Drug Class: unknown

Indication: epilepsy

Clinical formulation: [redacted] 250, 500, 750 mg

Route of administration: oral

Proposed clinical protocol or Use: adjunctive therapy in the treatment of partial onset seizures, with and without secondary generalization, in adults with epilepsy.

Previous clinical experience: 87 clinical studies conducted over 18 years.

Introduction and drug history: Levetiracetam is a new chemical entity and the active ingredient of Kepra tablets. Levetiracetam is the S-enantiomer of a-ethyl-2-oxo-1-pyrrolidine acetamide and is also referred to as ucb L059 or ucb 22059. It is chemically related to piracetam, a compound used in Europe for over 25 years for the treatment of various cognitive disorders. The intended use is in adult epileptic patients, as adjunctive therapy in refractory partial onset seizures with and without secondary generalization. The recommended dose range is 1000 to 3000 mg/day in a b.i.d regimen i.e. 20 to 60 mg/kg/day for a 50 kg patient. Many of the pharmacology/toxicology studies were previously reviewed in 1988 (Rosloff), 1993 (Rosloff), and 1994 (Freed) under several IND submissions. In animal studies, this drug was shown to have low toxic potential and a high therapeutic index.

Studies reviewed within this submission: pharmacology, safety pharmacology, immunotoxicology and genetic toxicology studies not previously reviewed; carcinogenicity studies; reproductive toxicity studies; 4 week study with up to 5% ucb L060 (degradant and enantiomer of ucb L059).

[redacted]

PHARMACOLOGY:

Thoroughly reviewed by Drs. L. Freed and B. Rosloff[redacted] A small number of pharmacology studies were conducted more recently and submitted in this NDA (see list of reviewed studies below). These were more animal models demonstrating anti-convulsant and anti-epileptic activity, also additional studies on mechanism of action.

Reviewed studies not previously reviewed:

1. Evidence for a unique profile of levetiracetam in rodent models of seizures and epilepsy, ADPE98H1303.
2. Anticonvulsant properties of ucb L059 (levetiracetam) in mice: comparison with currently prescribed and newly developed antiepileptic drugs, PDEE96K2507.
3. Assessment of pro-convulsant activity following single oral administration to mice, RRLE97G0706.
4. Levetiracetam (ucb L059) prevents limbic seizures induced by pilocarpine and kainic acid in rats, PDEE97C0603.
5. Effects of ucb L059, a novel antiepileptic drug, on convulsant activity in two genetic rat models of epilepsy, PDEE96B0901.
6. ucb L059, a novel anticonvulsant, reduces bicuculline-induced hyperexcitability in rat hippocampal CA3 in vivo, PDEE96A0201.
7. ucb L059 (levetiracetam) affects in vitro models of epilepsy in CA3 pyramidal neurons without altering normal synaptic transmission, ADPE98A2702.

Summary of Pharmacology:

The pharmacological activity of levetiracetam was evaluated in mice, rats, hamsters, guinea pigs and dogs. These studies revealed that levetiracetam displayed protection in various animal models of chronic epilepsy reflecting both partial and primary generalized seizures. There was no anticonvulsant activity in two screening tests for antiepileptic drugs (AEDs), the maximal electroshock (MES) test and the maximal pentylenetetrazol (PTZ) test. Levetiracetam lacked anticonvulsant action against seizures induced by maximal stimulation with different chemoconvulsants and showed minor anticonvulsant action with submaximal stimulation and also in threshold tests, one exception being the protection observed against seizures induced by pilocarpine and kainic acid. Seizure protection by levetiracetam was maintained after repeat administration (bicuculline and methyl-6.7-dimethoxy-4-ethyl-b-carboline-3-carboxylate in mice and rat). However, chronic treatment of amygdala kindled rats resulted in reduced seizure

protection. There was no rebound effect upon withdrawal of treatment with levetiracetam. Levetiracetam has anxiolytic properties and does not negatively impact cognitive function in mice and rats.

In vitro experiments, including ligand binding assays, have shown that the mode of action of levetiracetam is not due to any interaction with mechanisms of inhibitory and excitatory neurotransmission. Up to 1700ug/mL levetiracetam did not result in significant ligand displacement at known receptor binding sites. Glutamate receptor-mediated neurotransmission, second messenger systems, muscimol-induced chloride flux, ion channel proteins, gammabutyric acid-transaminase, and glutamate decarboxylase activities were unaffected by levetiracetam. A stereoselective binding site for the drug has been demonstrated in synaptic membranes from the CNS and not in peripheral tissue. Benzodiazepine receptor antagonists had no effect on levetiracetam protection against seizures.

SAFETY PHARMACOLOGY:

Previously reviewed by Drs. L. Freed and B. Rosloff [redacted] A few safety pharmacology studies were conducted more recently and submitted in this NDA (see list of reviewed studies below).

Reviewed studies, not previously reviewed:

1. Effects of cardiovascular and respiratory function in the anaesthetized Beagle dog (intravenous administration), RRLE97E1403.
2. ucb L059: Evaluation of effects on pulmonary arterial pressure and blood parameters following single intravenous administration in conscious dogs, RRLE98L1201.
3. Effect of ucb L059 on isolated rat pulmonary artery, RRLE98F0402.
4. Activity of ucb L059 on the isolated guinea pig ileum contracted by electrical stimulation, acetylcholine, histamine, nicotine, or serotonin, RRLE96B0501.
5. ucb L059. Assessment of effects on the isolated guinea-pig ileum, RRLE97A2403.

A previous reviewer (L. Freed) noted that the CV studies were only summarized in the IND and requested the study reports. The study reports were submitted in this NDA. The report results in the NDA agree with the summaries provided in the IND.

Summary of Safety Pharmacology:

Adverse **CNS effects** of levetiracetam were investigated in normal and kindled rat and mouse models. In normal rodents, levetiracetam produced moderate decreases in activity and hypotonia at oral or i.p. doses in the range 900-1800 mg/kg in observational tests for neurobehavioral effects (Irwin) and open-field observation. The effects showed a slow progression with increases in dose and were sometimes preceded by transient increases in activity. The performance on the rotarod was either unaffected up to 1700 mg/kg in rats and mice (i.p.) or transiently decreased at 1700 mg/kg and above (mice via gavage). Chimney climbing was impaired from 540 to 1700 mg/kg i.p. in rats and mice. The low potential of levetiracetam to produce CNS adverse effects was confirmed in corneally kindled mice by comparing protection against seizures and rotarod performance (see table 1. below) The absence of notable sedation in the dose-range of 100-1800 mg/kg (p.o.) was

confirmed by the absence of potentiation of pentobarbital sleeping time in rats, although earlier studies had provided conflicting results. Levetiracetam had no analgesic effect in the acetic acid writhing test. The physical dependence potential of levetiracetam was evaluated in rats at doses up to 1800 mg/kg/day for 40 days. Levetiracetam exposure followed by rapid termination did not produce behavioral signs of withdrawal. Additionally, levetiracetam administered to morphine control animals and naloxone administered to levetiracetam treated rats did not precipitate withdrawal.

Table 1. Comparative safety margin for CNS effects in kindled mice

Compounds	Impairment of rotarod performance	Protection against generalized kindled seizures	Safety margin
	TD50 (mg/kg i.p)	ED50 (mg/kg i.p.)	TD50/ED50
Levetiracetam	1036	7	148
Valproate	184	66	3
Phenobarbital	25	12	2
Clonazepam	0.08	0.03	3
Carbamazepine	33	6	6
Phenytoin	99	6	17
Vigabatrin	1464	210	7
Lamotrigine	29	4	7
Gabapentin	862	55	16

Effects on **cardiovascular and respiratory** functions were investigated in studies in awake or anesthetized rats and dogs in which levetiracetam was administered as an i.v. bolus or £ 5 min infusion. In anesthetized dogs, levetiracetam had minimal effects on blood pressure and heart rate up to 100 mg/kg i.v. At higher doses, it produced a rapid and transient decrease in blood pressure and aortic flow and increase in heart rate. From 1000 mg/kg i.v., tachycardia and AV block were observed and lethality occurred at 3200 mg/kg. In anaesthetized or awake dogs, a rapid (tmax : 5 min) and short-lasting (< 20 min), dose-related increase in pulmonary artery pressure was observed from 50 to 450 mg/kg i.v. In vitro, levetiracetam was shown to markedly increase blood and plasma viscosity and to decrease erythrocyte deformability at final concentrations of ≥ 7 to 144 mg/ml. These concentrations encompass the range of concentrations of the solutions injected intravenously. In awake dogs, rapid and transient increases in heart rate and diastolic blood pressure were also observed, mainly from 100 mg/kg i.v. (possibly related to emesis which was consistently seen at ≥300 mg/kg i.v.) No effects of levetiracetam were observed on heart rate or blood pressure responses to isoproterenol, epinephrine, norepinephrine, carotid occlusion, acetylcholine, peripheral vagus stimulation, histamine or 5-HT in anaesthetized dogs or on noradrenergic or KCl contractions in isolated rat aorta. No contractile effect of levetiracetam was observed on isolated rat pulmonary artery up to 30 mM (5.1 mg/ml). No effects were observed on ECG, heart weight and histopathology in the toxicity studies in the dog at doses up to 1200 mg/kg/day for up to 1 year (Cmax: 733 µg/ml, Cav: 241 µg/ml, i.e. 13 and 9 times the corresponding human values) following repeat 3 g/day doses. No histopathological changes were observed in the heart in the toxicity studies in the rat.

Hematological effects of levetiracetam were studied. Several studies were conducted *in vitro* and *ex vivo* to evaluate effects on platelet aggregation and coagulation. Inhibitory effects on collagen, but not ADP induced aggregation of platelets were observed at high concentrations (> 0.85 mg/ml). There were no effects observed in vivo, except for a small prolongation of tail bleeding time in rats (85 mg/kg p.o.). No effect was noted on bleeding of superficial brain arteries in rabbits up to 54 mg/kg i.v. and in toxicity studies (2- and 13-week studies in dogs).

Effects on **gastrointestinal** function were assessed *in vitro* on isolated guinea-pig ileum, on gastric secretion in pylorus ligated rats and on charcoal propulsion in mice (up to 540 mg/kg p.o.). Overall, levetiracetam had no significant effects. **Other** effects: single oral doses of 5.4, 54 and 540mg/kg had no effect on urine volume, electrolytes and protein excretion in male Wistar rats. Levetiracetam produced a small, not dose-related decrease (<1.5°C) in body temperature in the dose-range of 300-1800 mg/kg (oral) or 50-1700 mg/kg (i.p.) in rats and mice.

Conclusions:

There were no significant adverse effects detected in the animal safety pharmacology studies with levetiracetam. There appears to be a relatively wide safety margin for any adverse CNS effects with levetiracetam relative to other anti-epileptic drugs (AEDs).

PHARMACOKINETICS/TOXICOKINETICS:

Thoroughly reviewed by Drs. L. Freed and B. Rosloff[redacted]. A small number of pharmacokinetic studies (mostly *in vitro* studies) were conducted more recently and submitted in this NDA (see list of reviewed studies below). Numerous toxicokinetic studies were conducted (most were previously reviewed by Rosloff and Freed) in conjunction with the toxicology studies and, where appropriate, are discussed along with those studies in later sections of this review.

1. Preliminary investigations on the in vitro metabolism of ucb L059, RRLE97K2805.
2. In vitro metabolism of ucb L059 using human liver homogenates, whole blood and plasma, RRLE98B1803.
3. In vitro effects of ucb L059 on selected human liver microsomal CYP marker activities, RRLE95L2403.
4. In vitro effects of ucb L059 and progabide on human liver styrene oxide hydrolase activity, RRLE95J1303.
5. Effect of ucb L059 and ucb L057 on the in vitro glucuronidation of valproic acid, RRLE97D2201.
6. In vitro effects of ucb L059 on selected human liver glucuronidation marker activities, RRLE96K0301.
7. Characterization of P450 inhibition spectrum of ucb L059 and ucb L057, RRLE97D2202.
8. In vitro effect of ucb L057 on selected human liver drug metabolizing enzymes, RRLE97J0303.
9. Effect of ucb L059 and phenytoin on 7-ethoxycoumarin O-diethylase activity in primary cultures of rat hepatocytes, RRLE96K3107.

10. Effects of ucb L059 on the expression of various P450 in primary cultures of human hepatocytes, RRLE98F0502.
11. Pharmacokinetic evaluation plasma and brain levels. Interaction between ucb L059 and various anti-epileptic drugs in preventing audiogenic seizure in mice, RRLE96K3110.
12. Influence of a phenobarbital pre-treatment on the metabolic clearance of ucb L059 in the OFA rat, RRLE96C0603.

Summary of Pharmacokinetics/Toxicokinetics:

The pharmacokinetics (absorption, distribution, metabolism, and excretion) of levetiracetam were examined in the rat, mouse, rabbit, dog, and monkey following single oral, intravenous, or intraperitoneal. Repeat dose studies were also done in the rat and dog and in pregnant rats and rabbits. The potential for possible drug interactions was investigated *in vitro* (in human liver microsomes and human and rat hepatocytes) and in *in vivo* studies in mice and rats. In addition, drug monitoring was included as a component of several safety pharmacology studies and many of the toxicology studies.

Levetiracetam was rapidly (time to peak concentrations of approximately 1 hour), completely absorbed, and the extent of **absorption** was maintained over a large range of doses. However, the absorption rate, but not total absorption, was decreased in rats at doses ≥1200 mg/kg when given by gavage. In dogs, the extent of absorption was reduced at unit doses ≥600 mg/kg due to emesis.

Levetiracetam was rapidly **distributed** and tissue concentrations were close to those in blood after both single and repeat administration, with the exception of lower levels in the adipose tissue and lens and higher levels in kidneys, consistent with the renal excretion. The volume of distribution was approximately 0.5 to 0.7 L/kg. The exchange was slower for the CNS. Levetiracetam is not highly protein-bound (<10%). Levetiracetam crosses the placental barrier and enters the central nervous system (CNS).

The **metabolic pathways** across species are hydrolysis of the acetamide to form the carboxylic metabolite (ucb L057), hydroxylation of the oxopyrrolidine ring, opening of the ring in position 5, and combinations of these pathways. When administered directly, ucb L057 is primarily excreted unchanged in the urine. Acidic metabolites represent approximately 7 to 10% of the excreted dose in urine in rodents, and 20% in rabbits and dogs. The major circulating metabolite in man is ucb L057 which accounts for 24% of the dose excreted in urine, while the parent compound accounts for 66% (see Table 2 below). The extent of biotransformation is less in the rat than in the other species. In rabbits, the parent drug represents 60% of the dose in urine and ucb L057 and ucb K115 each accounts for 10% of the urinary radioactivity. After repeat administration at doses used in toxicity studies, the amount of metabolites resulting from ring hydroxylation or opening recovered in urine increased in the dog, and to a lower extent in male rats. In rats, the urinary excretion of minor metabolites, but not ucb L057, was increased by phenobarbital pretreatment. There was no evidence for enantiomeric interconversion of levetiracetam (the S-enantiomer) to ucb L060 (the R-enantiomer). The in vitro biotransformation of levetiracetam to the major human metabolite, ucb L057, is an enzymatic process with broad tissue distribution and does not involve cytochrome P-450s (CYP). Levetiracetam and/or its metabolites cross the placental barrier and there was no evidence for unexpected accumulation in

the fetus. At the pharmacologically active dose of 54 mg/kg, the pattern of distribution of levetiracetam and metabolites are similar for males and females, pregnant and nonpregnant animals, and after p.o. and i.v. administration.

Table 2: Species Comparisons of Urinary Metabolites Expressed as a Percent of Dose after a Single Oral Dose of 54 mg/kg 14 C-levetiracetam (Radioactive Profiles)

Parameter	Mouse	Rat	Dog	Man†
Sampling time	0 – 48 h	0 - 32 h	0 –24 h	0 - 48 h
Levetiracetam	61	72	62	66
ucb L057	5	5	8	24
ucb K115	3	1	6	1
3-OH, 4-OH metabolites	4	1	8	2
Others	11	4	4	1

† 500 mg p.o. in man

Urinary excretion is the primary route of **elimination**, with > 60% of an administered dose excreted as unchanged levetiracetam. In mass balance studies, the recovery was generally greater than 90% and there was no significant relevant retention of radioactivity in the tissues. Levetiracetam and its metabolites were almost completely excreted in urine. In all species a large proportion (60 to 85%) of the administered dose was excreted unchanged in the urine, a smaller proportion (7 to 30%) is excreted as metabolites. Urinary excretion of levetiracetam was overall independent of dose and duration of treatment or route of administration. Generally, less than 2% of the administered dose was excreted in the feces. Bile was not a significant route of elimination. Levetiracetam and its metabolites are excreted in the milk of lactating rats. The concentration of levetiracetam in milk was approximately 86% of plasma concentrations 1 to 6 hr after dosing.

Pharmacokinetic parameters calculated at several doses were similar in males and in females and overall the pharmacokinetics were linear. Pharmacokinetic data obtained from plasma and urine during toxicology studies indicate that the pharmacokinetics of levetiracetam is not significantly altered following repeated administration of toxicological doses. At the highest toxicological doses there is some blunting of the dose proportionality of exposure. See Table 3 (next page) for comparative pharmacokinetic parameters.

Table 3: Plasma Pharmacokinetic Parameters after a Single Dose of 54 mg/kg

Intravenous route

Parameter	Mouse	Rat	Rabbit	Dog	Man †
Half life (h)	1.3	2.2	3.9	3.3	7.7
Vd L/kg	--	0.67	0.53	0.71	0.69
AUCi.v. (µg.h/mL)	112	262	615	367	220
Clearance (mL/min/kg)	8.5	3.6	1.5	2.5	1.1

Oral Route

Tmax (h)	0.5	0.3	0.6	0.8	0.5
Cmax (ug/mL)	40	64	75	67	27.5
AUCpo (µg.h/mL)	98	270	613	403	231
Absolute Bioavail. % †††	88	100	100	110	106

† Single 1000 mg dose in man (20 mg/kg); ††AUCp.o./AUCi.v

Studies were conducted investigating the potential for pharmacokinetic drug interactions between levetiracetam and AEDs. In mice levetiracetam plasma levels (C1h) were decreased by 50% when coadministered with vigabatrin and plasma valproate levels were decreased by 33% when coadministered with levetiracetam. Brain/plasma concentration ratios of levetiracetam and AEDs were unaltered. Lower plasma concentrations of valproate were also observed after coadministration of levetiracetam (3000 mg/kg/day) in pregnant mouse. Levetiracetam did not alter phenytoin protein binding in vitro. Levetiracetam and its major human metabolite ucb L057 were extensively studied in human liver microsomes and showed no potential, up to 170 - 213 µg/ml, for inhibition of CYP isoforms, epoxide hydrolase, glucuronyltransferases and valproate glucuronidation. Levetiracetam did not induce liver enzymes in rat or human hepatocytes in culture although there is evidence for induction *in vivo* in animals.

TOXICOLOGY:

General Comments: A very useful table summarizing the drug substance batch numbers, dates of manufacture, and the manufacturing process (I or II) for all of the single and repeat dose toxicology studies, reproductive, carcinogenicity, genotoxicity studies and others is in Vol. 9, pages 121-14.

All of the important toxicology studies encompassed by this section were previously reviewed by B. Rosloff and L. Freed [redacted]. Carcinogenicity, reproductive toxicology and genetic toxicology are in later sections of this review. Several studies on two metabolites are reviewed here (genetic toxicology on these two metabolites is reviewed by Aisar

Atrakchi – see attachment). Metabolite L060 is a minor metabolite in humans and animals and was considered for development as a drug to improve cognitive function, and is also a degradant in the manufacturing process. Metabolite L057 is the major metabolite in humans and animals - levels were increased in patients with impaired renal function.

Study Title: 4-week oral toxicity study in the rat in the presence of levels of up to 5% ucb L060

Study No: TA0420, study report dated 7/98
Vol#: 71-72
Conducting laboratory and location: UCB Laboratory of Toxicology, Belgium
GLP compliance: yes
QA- Report Yes (X)
Dosing:

- species/strain: Sprague Dawley rats (Crl:CD (SD) BR), 6-7 weeks of age
- #/sex/group: 6
- satellite group for toxicokinetics: 3/sex/group (drug levels in urine).
- dosage groups in administered units: 4 groups, control, ucb L059 300 mg/kg/day, ucb L059 300 mg/kg/day with 1% ucb L060 (3 mg/kg), ucb L059 300 mg/kg/day with 5% ucb L060 (15 mg/kg).

Drug: ucb L060 batch 17, ucb L059 batch 136
Results:

- Clinical signs: no treatment-related abnormalities
- Body weights: no treatment-related abnormalities
- Food consumption: no treatment-related abnormalities
- Hematology: no treatment-related abnormalities
- Clinical chemistry: no treatment-related abnormalities
- Urinalysis: epithelial cell counts were mildly elevated in all male treated rats
- Organ Weights: no treatment-related abnormalities
- Gross pathology: no treatment-related abnormalities
- Histopathology: minimal centrolobular hepatocyte hypertrophy was increased in treated male rats with a similar incidence in all treated groups. Hyaline droplet nephropathy in treated male rat kidneys, similar incidence/severity in all treated groups
- Toxicokinetics: No ucb L060 (degradant in question) was detected in the urine of rats given ucb L059 alone. With the combination of ucb L060/059, urine excretion (% daily dose) of ucb L059 was the same as when given alone. Urine excretion (% daily dose) was the same for ucb L060 when given at 1% and 5% levels.

Key Study Findings: The addition of 1% and 5% ucb L060 (degradant in question) did not alter the toxicologic profile of ucb L059. The toxicologic findings are consistent with those from previous studies of ucb L059 (which in all animal studies/human studies has contained ucb L060 at or below the level of detection).

Study Title: ucb L057, 2-week range-finding oral toxicity study in the rat
Study No/Date: RRLE97F1102, UCB # TB0330, 1997
Conducting laboratory and location: UCB Laboratory of Toxicology, Belgium
Volume: #70
QA- Report: No / Non-GLP study
Dosing:

- species/strain: SD rat
- #/sex/group: 3
- satellite group for toxicokinetics: yes, 6/sex/group
- dosage groups in administered units: 250, 500, 1000, 2000 mg/kg/day

Drug: batch # A005
Results: No drug related mortality, no treatment related effects on food consumption, clinical signs, body weights, hematology, clinical chemistry, opthalmoscopy, and organ weights. Gross findings included distended ceca with yellow contents at 2000 mg/kg (both sexes) and 1000 mg/kg (females only). Histopathology – minimal epithelial hypertrophy of the ceca in all treated groups (similar incidence/severity in all treated groups, both sexes). Toxicokinetics – plasma levels linearly proportional to the dose. See table 4 below. Exposures in the highest dose group exceed the exposure at the highest human dose by factors of 40x (Cmax) and 11x (AUC).
Key Study Findings: No adverse effects in this non-GLP study with the major metabolite.

Study Title: ucbL057: range-finding study in the Beagle dog by oral gavage administration
Study No/Date: RRLE98A3001, UCB # TA0353, March-April 1997
Conducting laboratory and location: [redacted]
Volume: #70
GLP compliance: yes
QA- Report Yes (X)
Dosing:

- species/strain: beagle dog
- #/sex/group: 2
- dosage groups in administered units: 25, 100, 400 mg/kg/day

Results: No mortality. No treatment related effects on food consumption, clinical signs, body weights, hematology, clinical chemistry, opthalmoscopy, urinalysis, organ weights, gross and histopathology. Toxicokinetics – exposure linearly related to dose. Rapid and complete absorption. See table 4 below (next page). Exposures in the highest dose group exceed the exposure at the highest human dose by factors of 69x (Cmax) and 27x (AUC).
Key Study Findings: No adverse effects in this range-finding study.

Table 4: Comparative Plasma Pharmacokinetic Parameters of Metabolite ucb L057

Rat (Day14 of 14-day study, means of male and female values combined)

Dose (mg/kg/day)	T_{max} (hour)	C_{max} (ug/mL)	AUC_{0-24hr} (ug.h/mL)
250	1.0	42	96
500	1.0	59	165
1000	1.0	83	291
2000	1.5	119	618

Dog (Day 14 of 14-day study, means of male and female values combined)

Dose (mg/kg/day)	T_{max} (hour)	C_{max} (ug/mL)	AUC_{0-24hr} (ug.h/mL)
25	--	14	72
100	--	45	262
400	--	203	1558

Human (Day 7 of repeat dose study, healthy volunteers, Study N202)

Dose (mg/kg/day)	T_{max} (hour)	C_{max} (ug/mL)	AUC_{0-48hr} (ug.h/mL)
1000 mg b.i.d.	4.5	2	35
1500 mg b.i.d.	4.5	3	57

Overall Toxicology Summary:

The toxicity of levetiracetam was investigated in mice, rats, dogs and monkeys following single administration by the intravenous and/or oral routes. Subacute and chronic toxicity was evaluated in the rat (gavage) and dog (gavage or capsules). Additionally, dietary administration studies were conducted in mice and rats for dose selection and for carcinogenicity evaluation.

In single dose studies, i.v. administration of levetiracetam produced deaths within minutes of dosing at doses > 750 mg/kg in the mouse and rat; deaths were preceded by convulsions, decreased motor activity, ataxia, tachypnea, cyanosis, or side lying. No deaths occurred in dogs up to 1200 mg/kg i.v.; however, transient clinical signs of increased heart rate, restlessness, nervousness, emesis and unsteady gait were observed. Oral doses up to 5000 mg/kg/day in the mouse and rat were not lethal and were associated with only transient clinical signs (decreased motor activity, ataxia, tachypnea, side lying, proneness, unsteady gait, loss of righting reflex, and ptosis). Pharmacokinetic data indicated complete absorption of an oral dose; therefore, the difference in mortality indices between i.v. and p.o. administration may be related to peak plasma concentrations. Single dose escalation to 2000 mg/kg p.o. in monkeys was not lethal and resulted in transient behavioral observations indicative of CNS depression and emesis at ≥1000 mg/kg.

Levetiracetam has low toxicity as daily oral doses up to 1800 mg/kg in rats, 960 mg/kg in mice, and 1200 mg/kg in dogs administered for up to 1 to 2 years, produced no lethality, no organ failure, no irreversible toxicities or proliferative changes. Mortality in repeat dose studies was observed only following i.v. administration of 900 mg/kg/day to rats in a 2-week toxicity study. In all studies, clinical signs were minimal with the most consistent observations across studies

and species being neuromuscular effects and dose-related incidence and severity of post-dose salivation/wet coats. Generally, clinical signs were more prevalent following i.v. administration compared to oral gavage and less with dietary admixture. Decreased body weight gain, in conjunction with slightly decreased food consumption, was also observed in rats in the drug-in-diet studies but not in the gavage studies.

In rodents, the liver (mouse and rat) and the kidney (male rat) were the main target organs. Liver findings of increased liver weight and hypertrophy of centrolobular hepatocytes, associated with proliferation of smooth ER, were observed in the rat in repeat administration studies at doses ≥300 mg/kg/day by gavage and ≥50 mg/kg/day in the dietary studies. This change was more marked in males than in females and was reversible upon cessation of levetiracetam administration. Similar changes were seen in the mouse at 960 mg/kg/day. Proliferation of the smooth endoplasmic reticulum is usually associated with induction of microsomal enzymes, an adaptive response not generally considered an adverse effect of treatment. A consequence of these changes is confirmed by the small (2 fold) increase in plasma AST, ALT and alkaline phosphatase at 1800 mg/kg/day in the chronic studies. In addition, *in vitro* studies evaluating the effect of levetiracetam on enzyme activity in rat and human liver fractions indicate levetiracetam is neither a high affinity substrate nor inhibitor of liver metabolic enzymes. Levetiracetam did not increase CYP-supported activity or CYP proteins following 72-hour incubation of rat and human hepatocytes at 170 mg/mL. Centrolobular hepatocellular vacuolation, corresponding to neutral lipid deposition, was seen in male rats only and at doses ≥160 mg/kg/day and in mice at doses ≥240 mg/kg/day. This was also reversible following withdrawal of treatment and is considered of minor toxicological importance. No necrotic or proliferative changes in the livers of rats or mice were observed. *In vitro* levetiracetam was devoid of cytotoxicity in primary cultures of rat hepatocytes at concentrations up to 170 mg/mL.

Kidney pathology only occurred in male rats and consisted of hyaline droplet nephropathy which was associated with an increase in severity of chronic progressive nephropathy, occurrence of granular casts and of mineralization of the medulla, which are anticipated consequences. However, there was no evidence of severe, end-stage disease even after a 2-year exposure or on the incidence or occurrence of renal tumors. The incidence and severity of hyaline droplet deposition and chronic progressive nephropathy were increased in male rats treated with ≥50 mg/kg/day. The features of hyaline droplet nephropathy were dose-dependent, progressed with duration of dosing and regressed with termination of dosing; sequelae (chronic progressive nephropathy, granular casts and mineralization) did not regress. This male rat specific pathology observed at all doses in all of the repeat administration studies was demonstrated to be associated with alpha 2-microglobulin accumulation in the proximal tubules. A dose-dependent increase in the number, size and distribution of intracytoplasmic eosinophilic inclusions and renal alpha 2-microglobulin concentration was observed following administration of levetiracetam to rats. In addition, levetiracetam inhibited (IC_{50} of 25 mM) enzymatic degradation of renal alpha 2-microglobulin. These data indicate levetiracetam is one of the agents that induce male rat specific hyaline droplet nephropathy, which is not considered to be toxicologically relevant to man. There were no pathological changes in the kidney of females rats.

In the dog, transient behavioral signs (vomiting, nausea, neuromuscular effects, salivation, and unsteady/stiff gait) were observed only during the first few days of

administration at the highest dose levels (≥ 600 mg/kg/day). Liver and kidney weights were slightly increased, but no target organ was identified by histopathology. At 1200 mg/kg/day, alkaline phosphatases remained higher than in controls for up to one year and returned to control levels upon withdrawal of treatment. These changes, together with the increased urinary excretion of hydroxylated metabolites observed in the 2- to 13-week studies, are possibly indicative of liver metabolizing enzyme induction.

The previous reviewer (L. Freed) requested a GLP statement for the 3-month dog study. No GLP statement was submitted with this study in the NDA submission. This was a non-GLP study, conducted in the spirit of GLP as best this reviewer can assess from reviewing the protocol and study report. The results were very similar to a 52-week GLP dog study. This reviewer (J. Burris) accepts the 3-month non-GLP study as an acceptable and valid study.

The previous reviewer (L. Freed) seemed uncertain of the relationship between male rat renal pathology and alpha-2-microglobulin induced by UCB L059. This reviewer (J. Burris) has no doubt that this drug induces classic alpha-2-microglobulin nephropathy in male rats. L. Freed also questioned a possible relationship between glomerulonephrosis (synonyms glomerulonephropathy, rat chronic progressive nephropathy, CPN) and UCB L059. This reviewer has no doubt that the CPN noted in both sexes was NOT drug-related. The severity of CPN in chronic studies in male rats was increased secondary to the induction by L059 of alpha-2-microglobulin nephropathy. L. Freed also questioned the significance of the finding in rats of crystals in the urine and asked the sponsor to determine the nature and pathogenesis of these crystals. No information on crystals in the urine was provided in the NDA. This occurred in one study at one time point in females only (week 26 in the 52-week rat study). It was not noted in any other study in any other species or sex. This reviewer (J. Burris) concludes that this finding was of no toxicologic significance.

MOUSE CARCINOGENICITY:

Study Title: Potential tumorigenic effects in prolonged dietary administration to mice
Study Number: RRLE91K0702, UCB 293 91890
Volume Numbers: 53-57
Test Facility: Huntingdon Research Centre, Huntingdon, England
Study Date(s): treatment initiated 29 Aug 89, necropsies completed 20 Mar 91 (80 week study)
Date of Submission – previously submitted with IND (1994) but not reviewed
GLP Compliance/Quality Assurance: Yes (confirmed by UK GLP Monitoring Authority audit)
QA Report- Yes (X)
Study Type: carcinogenicity, oral admixed in the diet
Species/strain: mouse/Crl:CD-1 (ICR) BR
Number of animals per group; age at start of study: 52/sex/group; 3-4 weeks old
Animal housing: 4/cage (sexes separate), solid plastic cages with sawdust bedding
Drug Lot/Batch number(s): 900
Drug Purity / Stability / Homogeneity: Stable at room temperature (in darkness) for three years. Fresh mixtures for the diet prepared every two weeks. White crystalline powder, 98.1% pure. Homogeneity/concentrations verified prior to and during the study at 3 month intervals.

Doses: 0, 0, 60, 240, 960 mg/kg/day

- Basis of Dose Selection: 13-week study in mice (report UCB #311/891682, vols. 28-9). 15/sex/group, same strain, doses 0, 60, 240, 960, 3840 mg/kg/day. No treatment related mortality. Reduced body weights in both sexes at 3840 mg/kg (M 7%, F 9%) and as well as females at 960 mg/kg (7%). Reduced body weight gains in both sexes at 3840 mg/kg/day also in females at 960 mg/kg/day. Normal food consumption but reduced efficiency of food utilization (food intake per unit gain in body weight) in 960/3840 mg/kg females. Increased liver weights in both sexes at 3840 mg/kg/day also in males at 960 mg/kg/day. Increased incidence of centrolobular hepatocyte enlargement and vacuolation in both sexes at 240, 960, and 3840 mg/kg/day (moderate at 3840, minimal at other doses). NOAEL 240 mg/kg/day. Highest dose selected for carcinogenicity bioassay 960 mg/kg/day based on body weight reductions and liver pathology.
- Relation to Clinical Use: Repeat dose AUC in patients are 273 and 640 ug.h/mL at the recommended low and high doses of 20 and 60 mg/kg day. AUCs in mice at 78 weeks were (M/F average) 65, 314, 1505 ug.h/mL respectively for the dose groups 60, 240, 960 mg/kg/day. Mouse exposure in the highest dose group relative to the human low/high dose is 5.5X/2.4X.
- CAC Concurrence: CAC for protocol review did not exist when this study was initiated.
- Route of Administration: oral admixed in diet
- Frequency of Drug Administration: continuous in diet
- Dual Controls Employed: Yes, two control groups (groups 1,2), each 52/sex
- Interim Sacrifices: None
- Satellite PK or Special Study Group(s): None
- Unscheduled Sacrifices or Deaths: Occurred, of course, however no apparent relation to treatment and not unreasonable losses.

Study Results:

- Clinical Observations: Dose related increased incidence of yellow urogenital staining in mid and high dose groups of both sexes.
- Mortality: Mortality was unaffected by treatment. Survival was more than adequate in all groups to week 80 (avg. survival 67% males, 83% females).
- Body Weight: Body weights (group means) for the male high dose group were consistently depressed from week 5 throughout the study (approximately 3-5%). The male high dose group also had depressed body weight gains (21% when compared to combined controls) over the first 26 weeks. The male mid dose group had depressed body weight gains (33%) over the latter two-thirds of the study (weeks 26-80). Body weights (group means) for all female groups were similar throughout the study. All female treated groups had increased body weight gains (13-25%) in the first 26 weeks but depressed body weight gains (13-25%) from weeks 26-80 of the study. Weights were recorded weekly.
- Food Consumption: No treatment related effects. Measured weekly.
- Hematology: High dose groups, both sexes, had statistically significant decreases in

total WBC counts, neutrophils and lymphocytes at week 80. These were well within normal ranges and of no biological significance. Blood samples (orbital) collected at week 52 (not analyzed) and at termination, as well as at any interim euthanasia.

- Organ Weights, Clinical Chemistry, Ophthalmology: Not done.
- Gross Pathology: Dose related fur staining in the genital region (apparently, the same "yellow urogentital staining" noted during clinical observations) in all male dose groups and in the high dose female group. Dose related distention of seminal vesicles in male mid and high dose groups.
- Histopathology: Done on all indicated tissues (see tissue list below) of all control and high dose mice. Also, tissues of low and mid dose mice which died during the study. Done only on selected tissues of low and mid dose mice at termination (livers and gross lesions).

Non-Tumor – Increased incidence and severity of moderate to marked centrolobular hepatocyte enlargement in the high dose male group. Increased incidence of minimal to moderate centrolobular hepatocyte vacuolation in the high dose male group. Individual high dose males with centrolobular hepatocyte vacuolation also had centrolobular hepatocyte enlargement. Increased incidence of centrolobular hepatocyte vacuolation but not enlargement in mid and high dose females. The clinical and gross abnormalities of urogenital staining and distended seminal vesicles had no microscopic correlates.

Tumor – No treatment related increased incidence of neoplasms or pre-neoplastic lesions. No unusual neoplasms. No decreased tumor latency or enhancement of tumor progression.

- Toxicokinetics: Blood for drug level assays collected at week 78 from 20/sex/group and at week 80 from all remaining animals. Study # RRLE91K2201 "Plasma concentrations of ucb L059 in mice following repeated oral dietary administration." Blood samples from mice in this carcinogenicity study obtained at week 78 from 10/sex/group and at study termination from all available mice (then frozen, sent to Belgium for analysis). In treated groups, plasma concentrations in males exceeded females (approximately 1.5:1), plasma concentrations approximately linearly related to the nominal doses in both sexes. No apparent accumulation (in plasma) with chronic administration. No drug was detected in week 78 samples from control groups (two control groups, each 52 animals). At terminal sacrifice (week 80) low drug levels were detected in a small number of control group 1 animals of both sexes (6/35 males, 6/40 females, total 12/75). Of these twelve animals, levels in ten were less than 0.50 ug/ml, the lowest limit of reliable detection, two animals had levels at 1.10 and 0.59 ug/ml). Diet analysis throughout the study was uneventful. Study report concluded that contamination, most likely during weekly formulation and/or admixture of pre-mix, had occurred "at the end of the study." The possibility that these errors occurred at other points throughout the study cannot be dismissed which may have resulted in contamination of control diet with drug throughout the study.

Overall Interpretation and Evaluation:

- Adequacy of the carcinogenicity studies and appropriateness of the test model:

Appropriate route. Species acceptable, however relative exposures are much lower

for the mouse than for other animal species (see Table 3, page 8). NOEL was 60 mg/kg/day based upon microscopic findings in the liver and depressed body weights/body weight gains. Did not achieve an MTD in either sex however the dose levels were selected appropriately based upon body weight reductions and liver pathology in the 13-week study i.e. body weights reduced 7/9% in M/F at 3840 mg/kg, and 7% in F at 960 mg/kg; increased liver weight in both sexes at 3840 mg/kg and in males at 960 mg/kg; moderate centrolobular hepatocyte enlargement and vacuolation in both sexes at 3840 mg/kg, minimal at other doses. Very close to an MTD in males on this study (high dose males had body weights reduced 3-5% throughout the study, moderate to marked centrolobular hepatocyte enlargement). No MTD achieved for the females in this study. Due to good survival, the study could have been extended, however the protocol dictated termination at 18 months and 18-month mouse studies were common at the time (1989). The possible contamination of control diet with drug did not affect the validity of the study since this occurred randomly, at very low levels, and in small numbers of animals.

- Evaluation of Tumor Findings: No positive findings, no borderline findings, no suggestive findings of a possible tumor effect.
- Statistical Reviewer Comments: Statistical evaluation agrees with that in study report. No tumor findings reached statistical significance. Questions validity of protocol that terminated the study at 80 weeks despite excellent survival. Agrees that male high dose very close to an MTD but not females.

Summary /Conclusions:

- Acceptability of Study(s) or Overall Testing Approach:

The CAC concluded that this was not an acceptable study. From the minutes: *"The committee agreed that the mouse study was unacceptable, despite negative tumor findings, primarily because there was no appropriate basis for dose selection i.e. an MTD was not achieved, body weights were not significantly reduced, no toxic endpoints were evident, and exposures only exceeded humans by 2-5X. In addition, by current standards the study should have been extended to 104 week as survival was good at week 80."*

This reviewer (J. Burris) disagrees with the conclusion of the CAC.

"No appropriate basis for dose selection:"

Based upon the results of the 13-week study in the mouse, the only repeat dose study done in the mouse prior to the carcinogenicity study, dose selection for the carcinogenicity study was appropriate. In the 13-week study, body weight reductions and liver pathology indicated that the 3840 mg/kg dose in both sexes would be too high for an 18-month study. Also, body weight reductions and liver pathology at 960 mg/kg in the 13-week study indicated significant toxicity would very likely be evident at this dose in the 18-month study. Body weights were reduced 7/9% in M/F at 3840 mg/kg and 7% in females at 960 mg/kg, with increased liver weights in both sexes at 3840 mg/kg and in

males at 960 mg/kg, also with moderate centrolobular hepatocyte enlargement and vacuolation in both sexes at 3840 mg/kg which was minimal at other doses.

"An MTD was not achieved:"
Unfortunately, the results of the 18-month study did not correlate well with the predictions made based upon the 13-week study. The high dose 960 mg/kg males had body weights reduced only 3-5% throughout the study with moderate to marked centrolobular hepatocyte enlargement. The high dose 960 mg/kg females had no reduction in body weights and minimal centrolobular hepatocyte enlargement. In retrospect, the dosing in the carcinogenicity study could have been significantly higher in the females and possibly higher in the males, though probably not much higher in the males. An MTD was nearly achieved in the males based on body weight/liver pathology. I agree with the CAC that no MTD was achieved in the females.

"Body weights were not significantly reduced:"
Unfortunately, the results of the 18-month study did not correlate well with the predictions made based upon the 13-week study. The high dose 960 mg/kg females had no reduction in body weights in the carcinogenicity study, though body weights in females at this dose in the 13-week study were reduced 7%. The high dose 960 mg/kg males in the carcinogenicity study had body weights reduced 3-5% throughout the study. This may not be the desired ballpark figure of 10%, but it is a positive treatment-related effect and a real reduction in body weight. Males at this dose in the 13-week study had no body weight loss.

"No toxic endpoints were evident:"
There were no toxic endpoints discovered for this drug in dogs, rats, or mice other than liver pathology in rodents (which are adaptive changes due to treatment) and alpha-2-microglobulin nephropathy in male rats. There is no reason to suspect that the mouse carcinogenicity study could identify a new toxic endpoint, not discovered in all previous studies, by increasing the dose.

"Exposures only exceeded humans by 2-5X:"
True. The basis of dose selection was not exposure.

"By current standards the study should have been extended to 104 weeks as survival was good at week 80:"
I agree with the CAC that the study could have been extended beyond 18-months as survival was excellent. However, the protocol was simply to end the study at 18-months as was commonly done in mouse studies at the time. I would not reject an 18-month mouse study done ten years ago unless there was some indication that treatment related tumors were occurring late in the study, which is not the case in this study.

In summary, this reviewer disagrees with the CAC and accepts the mouse study. Despite its deficiencies, dose selection was appropriate and based upon the results of a 13-week study though retrospective criticism is warranted due to the apparent lack of predictive power of the 13-week study for the 80-week study. The lack of any borderline or positive

tumor findings in this study strengthens the acceptability of the study. The male part of the study was acceptable as a near-MTD was clearly achieved. The female part of the study could be repeated, if the Division deems this appropriate, as the dose could have clearly been higher.

- Major Tumor Findings: None
- Non-neoplastic Findings: Reduced body weights and body weight gains in male high dose group. Liver pathology (centrolobular hepatocyte hypertrophy/vacuolation) in high dose groups (M>F) probably adaptive however toxicity/adversity of these findings are difficult to evaluate with no clinical pathology/organ weight data.

RAT CARCINOGENICITY:

Study Title: Potential tumorigenic and toxic effects in prolonged dietary administration to rats
Study Number: UCB 292/91998
Volume Numbers: 57-63
Test Facility: [redacted]
Study Date(s): June 1989 (first dosing) - June/July 1991 (weeks 104-105)
Date of Submission: previously submitted with IND (1994) but not reviewed
GLP Compliance/Quality Assurance: Yes (confirmed by UK GLP Monitoring Authority audit)
Study Type: carcinogenicity, 104 weeks
Species/strain: Crl:CD (SD) BR rats
Number of animals per group: Four groups, 50/sex/group (main) and 20/sex/group (satellite for blood/interim sacrifice terminated at 52 weeks)
Animal housing: 5/cage
Drug Lot/Batch number(s): 900
Drug Purity / Stability / Homogeneity: Stable at room temperature (in darkness) for three years. Fresh mixtures for the diet prepared every week. White crystalline powder, 98.1% pure. Homogeneity/concentrations verified prior to and during the study at 3-month intervals.

Doses: 0, 0, 50, 300, 1800 mg/kg/day

- Basis of Dose Selection: UCB #227/87415 52-week toxicity in rats by oral gavage. 70, 350, and 1800 mg/kg/day. (Reviewed in '94). No effects on survival, body weights, food consumption. Liver and kidney target organ toxicity in males and females at 1800 mg/kg included increased weights, gross enlargement, centrolobular hepatocyte enlargement/vacuolation, progressive glomerulonephritis with renal tubular cytoplasmic eosinophilic inclusions (males only).
- Relation to Clinical Use: Repeat dose AUC in patients are 273 and 640 ug.h/mL at the recommended low and high doses of 20 and 60 mg/kg day. AUCs in rats at 102 weeks were (M and F averaged) 275, 1158, and 3864 ug.h/mL respectively for the dose groups 60, 240, 960 mg/kg/day. Rat exposure in the highest dose group relative to the human low/high dose is 4.2X/6.0X.
- CAC Concurrence: No CAC for protocol review existed at the time.
- Route of Administration: oral admixed in the diet
- Frequency of Drug Administration: continuous in the diet
- Dual Controls Employed: yes

- Interim Sacrifices: at 52 weeks (20/sex/group satellite for bleeding)

Study Results:

- Clinical Observations - Increased incidence of yellow urogenital staining in high dose groups, both sexes.
- Mortality: Control group 2 males and control group 1 females had increased mortality (56 and 58% respectively), mid and high dose groups of both sexes had reduced mortality (22 and 30 % males, 28 and 42% females) over the course of the study.
- Body Weight: mid and high dose groups of both sexes had greatly reduced body weights throughout the study (18 and 27% males, 20 and 33 % respectively). Body weight gains were significantly reduced in mid and high dose groups of both sexes in the first 52 weeks of the study.
- Food Consumption: Reduced in mid and high dose males (5 and 10% respectively) and in high dose females (5%). Achieved drug intakes were acceptably close to nominal levels.
- Ophthalmoscopy: No treatment related findings at weeks 52 and 103 (control group 1 and high dose groups only examined).
- Hematology: No treatment related findings (weeks 13, 26, 52, 78 and 104).
- Clinical Chemistry: Increased BUN, total protein, and globulin in high dose males. Increased GOT, GPT in high dose males and females.
- Urinalysis: Decreased pH in high dose males and females.
- Organ Weights: (adrenals, brain, heart, kidneys, liver, ovaries, pituitary, testes, thyroid weighed). Increased liver and kidney weight in high dose groups, both sexes. Increased kidney weight also in mid dose females. Decreased weights of testes/epididymes in all treated males.
- Gross Pathology: Increased incidence of urogenital staining of the hair in both high dose groups. Increased incidence of pale kidneys in high dose males.
- Histopathology: Done on control and high dose animals at interim and terminal sacrifice. Also, all animals from any group dying on study, all tissues from any group with gross abnormalities. Any tissue/organ in the high dose with an identified possible treatment related change was also examined in the low and intermediate groups. See tissue list below.

  Non-Tumor: Liver – minimal to moderate centrolobular hepatocyte enlargement with vacuolation in all treated groups (dose related increased incidence and severity in males; dose related increased incidence but not severity in females), minimal to moderate generalized hepatocyte enlargement only detected in high dose males, increased incidence of centrolobular hepatocyte fat in high dose males. Liver EM – smooth ER proliferation in centrolobular and periportal hepatocytes of high dose groups both sexes but not low dose groups (mid dose not sampled). Testes – dose related increased incidence of tubular atrophy in all treated male groups. Kidney – dose related increased incidence and severity of esosinophilic intracytoplasmic droplets/granules in cortical tubules in mid and high dose males, dose related increased incidence cortical tubular basophilia in mid and high dose males, medullary mineralization detected only in mid and high dose males with greater

incidence and severity at high vs. mid doses, corticomedullary junction mineralization only detected in high dose males, reduced incidence and severity of progressive glomerulonephritis (a.k.a. nephropathy) in mid and high dose groups of both sexes (probably related to reduce body weights in these groups).

Tumor: Body weight losses with increased survival in mid and high dose groups may have influenced tumor incidence. Reduced incidence of common tumors (pituitary adenomas, mammary fibroadenomas) in mid and high dose groups. No treatment related increased incidence of neoplasms or pre-neoplastic lesions. No unusual neoplasms. No decreased tumor latency or enhancement of tumor progression.

- Toxicokinetics: Study RRLE91M1101 "plasma concentrations of ucb L059 in rats following repeated oral dietary administration at various dosage levels" (study on the potential tumorigenic effects of ucb L059 to rats, UCB 292). Plasma concentrations lower in females than males in general. Plasma concentrations and AUCs approximately linearly related to the nominal dose up to 300 mg/kg. Plasma concentrations/AUCs were lower than would be expected at 1800 mg/kg. Reliably measured down to concentrations of 0.10 ug/ml. Low levels of drug were detected in both sexes from both control groups in small numbers of animals at all time points sampled. Week 52 control group 1, 2/10 animals at or below limit of detection (0.10 ug/ml). Week 52 control group 2, 2/10 animals at 0.20 and 0.50 ug/ml. Week 60 control group 1 (n = 4/10 animals) and control group 2 (n = 3/10 animals), 5 animals at or below the limit of detection, 2 animals at 0.20 and 0.30 ug/ml. Week 102, control group 1 had 8/40 tested with detectable drug levels (3 under 0.50 ug/ml, three at 0.60, one at 0.8, one at 1.0ug/ml. Week 102, control group 2 - only 1/40 (0.60 ug/ml). Example plasma concentration (group means) at 50, 300, and 1800 mg/kg dose respectively 12, 50, and 170ug/ml. Study report concludes that "there was some sporadic low contamination which occurred in a few samples from control animals and/or that some interferences were present in the samples." The possibility that some control animals (varying from 20% to 40%) had low levels of drug (generally at or below the limit of detection) in the diet at various time points throughout the study cannot be dismissed.

Overall Interpretation and Evaluation

- Adequacy of the carcinogenicity studies and appropriateness of the test model:

Appropriate route, though administration by gavage may have preferable and may not have resulted in the dramatic body weight losses. Appropriate species. NOAEL 50 mg/kg/day based upon body weights, renal and hepatic pathology. Large decreases in body weight in mid and high dose groups resulted in decreased mortality, reduced common tumor burden (pituitary/mammary adenomas), and reduced incidence/severity of common non-neoplastic conditions (progressive nephropathy). MTD exceeded at the mid and high doses based on body weights but not mortality (survival prolonged likely due to the reduction in incidence and severity of progressive nephropathy). The fact that some control animals (varying from 20% to 40%) had low levels of drug (generally at or below the limit of

detection) in the diet at various time points throughout the study leads one to question the validity of the entire study (as in the mouse study).

- Evaluation of Tumor Findings: No positive findings.
- Statistical Reviewer Comments (R. Kelly): Statistical evaluation agrees with that in study report. No tumor findings reached statistical significance. High dose exceeded the MTD based on body weight.

Summary Conclusions and Recommendations

- Acceptability of Study(s) or Overall Testing Approach: Acceptable (see attachment #4 CAC report). MTD was exceeded based on body weight but mortality decreased. This may have been due to poor palatability of the drug in the diet at the mid and high dose, however other rat drug-in-diet studies had no evidence of any palatability problems. Gavage would have been the preferred route of administration because gavage studies, as opposed to drug-in-diet studies, did not affect body weight and higher exposures may have been achievable. The tumor burden may have been favorably affected by the great decreases in body weight. Possible low level contamination of diet in some of the control animals is problematic, however the lack of any even borderline positive tumor findings indicates that this drug does not cause cancer in rats.

- Major Tumor Findings: None
- Non-neoplastic Findings: Decreased body weights and mortality in mid and high dose groups. Hepatotoxicity - increased GOT/GPT and liver weights in high dose males and females, generalized hepatocyte hypertrophy with centrolobular hepatocyte fat accumulation in high dose males. Renal toxicity - increased BUN with pale kidneys and increased kidney weights in high dose males, increased incidence and severity of α-2-microglobin in cortical tubules in mid and high dose males.

Table 5: Histopathology Inventory

Study	Carc.	Carc.		
Species	Mouse	Rat		
Adrenals	•	x		
Aorta				
Bone Marrow smear				
Bone (femur)	•	x		
Brain	• 3 areas	x 3 areas		
Cecum	•	x		
Cervix	•			
Colon	•	x		
Duodenum	•	x		
Epididymis	•	x		
Esophagus	•	x		
Eye	•	x		
Fallopian tube				
Gall bladder				
Gross lesions	•			
Harderian gland				
Heart	•	x		
Hyphophysis				
Ileum	•	x		
Injection site				
Jejunum	•	x		
Kidneys	•	x		
Lachrymal gland				
Larynx				
Liver	•	x		
Lungs	•	x		
Lymph nodes, cervical	•	x		
Lymph nodes mandibular				
Lymph nodes, mesenteric	•	x		
Mammary Gland	•	x		
Nasal cavity				
Optic nerves				
Ovaries	•	x		
Pancreas	•	x		
Parathyroid	•	x		
Peripheral nerve				
Pharynx				
Pituitary	•	x		
Prostate	•	x		
Rectum	•	x		
Salivary gland	•	x		
Sciatic nerve	•	x		
Seminal vesicles	•	x		
Skeletal muscle	•	x		
Skin	•	x		
Spinal cord	•	x		
Spleen	•	x		
Sternum	•	x		
Stomach	•	x		
Testes	•	x		
Thymus	•	x		
Thyroid	•	x		
Tongue	•	x		
Trachea	•	x		
Urinary bladder	•	x		
Uterus	•	x		
Vagina	•			
Zymbal gland				

MOUSE AND RAT CARCINOGENICITY – Study Audits by EMEA:

By letter dated July 16, 1999 (amendment #16), UCB Pharma informed FDA that the European Agency for the Evaluation of Medicinal Products (EMEA) suspended review of the licensing application for ucb L059. This action was taken pending audits of the above rat and mouse carcinogenicity studies (and the 13-week range finding study for dose determination in the mouse). This action was due to the presence of drug levels in plasma of some untreated control animals in all of these studies.

There were a number of other discrepancies/problems identified such as: seven tissues from seven rats (3 liver, 2 kidney, 1 pituitary, 1 uterus/cervix from control and low dose females/low and mid dose males) were reported as "normal" in the study report were reported as "missing" by the peer review pathologist, one additional study (immunostaining of male rat kidneys for α-2-microglobulin) had no protocol or amendment therefore not considered to be a GLP study, no abbreviation glossary in the study report, humidity during the study deviated from that in the protocol, and so on.

In an amendment to this NDA (#22) dated August 31, 1999, UCB Pharma provided an update on the status of this investigation which included the audit report by the UK GLP Monitoring Authority as well as [redacted] response to the audit report. The auditors found no GLP deviation which could account for the presence of test substance in the plasma of untreated animals however it could not be excluded that control animal had been exposed to the test substance due to mix-ups and contamination of feeds (or processing or analysis of plasma). The auditors discovered that the tissues read as "normal" by the study pathologist found to be "missing" by the peer review pathologist had not been read by the study pathologist and had never been processed – this was regarded as a significant deviation from GLP. The UK auditors concluded that, despite some deficiencies, overall this study had been conducted in compliance with the Principles of GLP. [redacted] compared control animal tumor incidence data in these studies to data from other studies at their lab and found no differences (however no comment was made if these other studies did or did not have drug detected in control animal plasma).

In an amendment to this NDA (#24) dated 30 September1999, UCB informed us that the EMEA had completed their audits and lifted their suspension of review of the levetiracetam licensing application. This submission included 1. an independent audit by EPL of UCB's analytical lab in Belgium (where the plasma samples for the carcinogenicity studies were analyzed), 2. an audit of this facility by the Belgian GLP Monitoring Authority, 3. UCB's response to the Belgian auditors, 4. several amendments to the study reports that address minor deviations noted by the UK GLP auditors, and 5. a consensus document issued by the independent peer reviewer and the Huntingdon pathology manager regarding the results of the pathology peer review of the rat carcinogenicity study.

The Belgian auditors found no explanation for the presence of detectable drug levels in the plasma of control animals and found "only minor deviations from GLP that did not jeopardize the integrity of the data." EPL auditors (requested by UCB) came to the same conclusions. An independent peer review of slides from the rat study was conducted (all tumors in all groups, all

tissues from 7 control and high dose males and females, all tissues from 5 decedents per sex in the low and intermediate groups, and the 7 missing slides i.e. originally reported as normal but actually were missing and had not been processed). The Huntingdon pathology manager reviewed the original and peer review reports and examined representative slides. The peer reviewer and Huntingdon pathology manager reached consensus on pathology issues and released a consensus document. This consensus document reaffirms that that there was no effect of treatment on tumors in the rat study, and that the results from the missing slides did not alter the conclusions of the study. Amendments to the original reports address a number of minor deficiencies discovered by the UK auditors which did not affect the validity of the study.

Pending - UCB, independently, not at the request or provocation by the EMEA, is conducting an independent peer review of the entire rat carcinogenicity study and will repeat the statistical tumor analysis (expected completion in November 1999).

IMMUNOTOXICOLOGY:

Two immunotoxicity studies were conducted and are reviewed here. One was previously submitted but not reviewed, the other is more recent.

Study title: 4-week oral (gavage) toxicity study in the rat: effect on the immune system
Study No and date: RRLE93L2501, June 1995
Site and testing facility: [redacted]
GRP compliance: Yes
QA- Report Yes (X)
Methods:

- Species/strain: Sprague Dawley rats
- Doses employed: 0, 50, 300, or 1800 mg/kg/day p.o. once daily by for 4 consecutive weeks.
- Route of Administration: gavage
- Number of animals/sex/dosing group: 10/sex/group
- Observations: In addition to standard clinical and histopathological examinations, the following parameters were measured: IgM and IgG; iliolumbar lymph node, spleen and thymus weight; histology of these tissues as well as other lymph nodes, Peyer's patches and bone marrow; spleen cellularity and cell viability; natural killer cell activity of spleen cells, lymphocyte subset count in spleen, phagocytosis by peripheral blood leukocytes.
- Results: Treatment-related salivation, staining of the fur, and lacrimation were noted at 300 mg/kg/day and higher. There were no deaths or treatment-related effects on body weight, food consumption or clinical pathology parameters. At terminal sacrifice, kidney and liver (males only) weights were increased in 1800 mg/kg/day animals. Histologically, intracytoplasmic eosinophilic inclusions (hyaline droplets) were observed in the proximal convoluted tubules in the kidneys of males from all dosage groups. At 1800 mg/kg/day, this was accompanied by an increase in the number and severity of basophilic tubules and, in 4 males, with granular casts. These findings were considered to represent alpha-2-microglobulin nephropathy. In addition, all males and 5 of 10 females at 1800 mg/kg/day and 2 males at 300 mg/kg/day had minimal to slight centrolobular hepatocellular

hypertrophy. There were no treatment-related changes in immunoglobulin assay, spleen cell viability or number, lymphocyte subsets, functional evaluation of nonspecific immunity, or morphology of the immune tissue. The NOAEL was 50 mg/kg/day.
Conclusion: No effects on immune functions/tissues in this study in the rat.

Study title: Antigenicity study of ucb L059 in guinea pigs
Study No and date: RRLE96H2804, December 1996
Site and testing facility:
GRP compliance: Yes
QA- Report Yes (X)
Methods:

- Species/strain: male guinea pigs
- Tests: active systemic anaphylaxis (ASA, 5/group), passive cutaneous anaphylaxis (PCA, 10/group), and passive hemagglutination reactions.
- Observations: To test **ACA**, animals (5/group) were sensitized by administration of levetiracetam 600 mg/kg p.o. five times per week for 2 weeks or by 600 mg/kg s.c. in combination with Freund's complete adjuvant one time per week for 3 weeks. Clinical signs and body weight were monitored. Levetiracetam 600 mg/kg i.v. was administered as the challenge 14 days after end of sensitization. Another group of animals were administered ovalbumin (10 mg/kg s.c.) and subsequently challenged with ovalbumin. Animals were evaluated for anaphylactic symptoms for 60 minutes after administration of the challenge dose. **PCA** evaluation was conducted using dilutions of antisera obtained from sensitized guinea pigs (2 animals/antiserum). Antisera dilutions were injected intradermally into the shaved back. Four hours after passive sensitization, challenge solutions of levetiracetam 600 mg/kg with Evan's blue dye were injected i.v. Thirty minutes after challenge, guinea pig backs were evaluated for PCA reaction and PCA titers were determined. Sheep red blood cells (RBCs) were incubated with 200 mg/mL levetiracetam for 1 hour for use in the **hemagglutination test** with dilution of antisera from treated guinea pigs.
- Results: No ASA reactions were observed following administration of a challenge dose to orally or subcutaneously levetiracetam-sensitized guinea pigs. No PCA reactions were observed after challenge with levetiracetam in animals passively sensitized with antisera obtained from guinea pigs orally or subcutaneously sensitized with levetiracetam. No hemagglutination was noted with antisera obtained from ucb L059 oral or s.c. sensitization groups following addition of ucb L059 sensitized red blood cells. Also no hemagglutination of ucb L059 sensitized red blood cells was observed in antisera obtained from the saline sensitization group. Animals sensitized with ovalbumin produced the expected responses.

Conclusion: No antigenicity was demonstrated in tests in the guinea pig.

REPRODUCTIVE TOXICOLOGY:

Reproductive toxicity (parental and fetal) was evaluated in a number of studies in rats and rabbits. Fertility and general reproductive performance were evaluated in rats, effects on pregnancy and teratology were evaluated in rats and rabbits, and effects of peri-natal and post-natal exposure were evaluated in rats. Two studies were also performed in pregnant mice to

evaluate the effect of levetiracetam administered alone or in combination with valproate. The embryo-fetal effects of levetiracetam were evaluated in teratology studies performed in mice, rats and rabbits. In mice, two drug interaction studies were conducted with levetiracetam and sodium valproate. In rats, two nearly identical teratology studies were conducted. Three studies were performed in rabbits. The peri- and post-natal effects of levetiracetam administration were evaluated in rats in a preliminary non-GLP and a confirmatory GLP study.

The following studies are reviewed:
1. A study of the effect of ucb L059 on fertility and general reproductive performance of the rat, RRLE91M0202
2. Oral teratological study in rats - Segment II, LE86L251
3. Study of the effect of ucb L059 on pregnancy of the rat, RRLE91K1802
4. Report on oral teratological study in rabbits – Segment II, LE85K301
5. A study of the effect of ucb L059 on pregnancy of the rabbit (UCB 382), RRLE91G0902
6. A preliminary study of the effect of ucb L059 on pregnancy of the rabbit RRLE92L2301
7. A study of the effect of ucb L059 on pregnancy of the rabbit (UCB 386), RRLE91G0902
8. ucb L059 and sodium valproate- dose range-finding teratology study in the mouse, RRLE98A3002
9. ucb L059 and sodium valproate: An interaction teratology study in the mouse, RRLE98F1501
10. A preliminary study of the effect of ucb L059 on the pregnant rat and offspring during the peri and post natal period - Segment III, RRLE91B0801
11. A study of the effect of ucb L059 on the pregnant rat and offspring during the peri and post natal period –Segment III, RRLE91G1001

Toxicokinetic studies to support reproductive toxicology:
12. Pharmacokinetic aspects in Sprague-Dawley rats, LE86H072
13. Pharmacokinetic aspects in New Zealand rabbits, LE86M031

Study title: Segment I -Reproductive Performance and Fertility- A study of the effect of ucb L059 on fertility and general reproductive performance of the rat
Study No/Date: RRLE91M0202, March 1992
Site and testing facility: [redacted]
GLP compliance: Yes
QA- Reports Yes (X)
Lot and batch numbers: 901
Protocol reviewed by Division No (X):
Methods:

- Species/strain: rat, Crl:CD (SD) BR
- Doses employed: 0, 70, 350, or 1800 mg/kg/day
- Route of Administration: p.o. by gavage
- Study Design: Male rats were administered levetiracetam for 9 weeks prior to pairing and through to termination (after weaning of the F_1 offspring). Female rats were dosed for 2 weeks prior to pairing through to termination either on Day 20 of pregnancy or after rearing their young to weaning. On Day 21 *post-partum*, 12 male and female offspring/group were selected to form the F_1 generation in which general and behavioral

development and reproductive capacity were assessed. The F 1 generation animals were exposed to levetiracetam only *in utero* or through the mother's milk.

- Number of animals/sex/dosing group: 30
- Parameters and endpoints evaluated: On Day 20 of pregnancy, 14 females/group were sacrificed and examined macroscopically; ovaries and uteri were examined. Fetuses were examined externally and preserved. Half the fetuses of each litter were processed for visceral examination and half for skeletal examination (unossified sternebrae).

Results:

- Clinical signs: In parental animals, post-dose salivation and reduced muscle tone at 350 and 1800 mg/kg/day.
- Mortality: None in adults. There was a slight increase in pup mortality from birth to Day 8 post-partum among litters born spontaneously to dams given 1800 mg/kg/day.
- Body weight: Body weight gain was lower between weaning and Week 4 among offspring (both sexes) in the F 1 generation from dams treated with 350 or 1800 mg/kg/day.
- Fertility and Embryonic Development: No effect on mating performance and fertility of the parental generation at doses up to 1800 mg/kg/day. At the highest dose, fetuses examined on Day 20 of gestation demonstrated a significant reduction in litter and mean fetal weight and an increase in the incidence of skeletal anomalies (reduced ossification and skeletal variants with unossified/reduced sternebrae). The reduced ossification was also observed in fetuses from the 350 mg/kg/day dose group. In fetuses delivered spontaneously on Day 22 of pregnancy, litter and mean fetal weight was similar in all groups.
- F1: Evaluation of the F1 generation revealed no effect on general behavioral development or reproductive performance. The mating performance, and duration of pregnancy in F1 rats, and litter data of their offspring up to weaning were similar in all groups.

Conclusions: No effects on mating performance or fertility of FO adults. Effects on their offspring noted at 350 and 1800 mg/kg (lower body weight gain, reduced ossification with skeletal variants).

Study title: A study of the effect of ucb L059 on pregnancy of the rat (segment II teratology)

Study No/date: RRLE91K1802, March 1992

Site and testing facility: [redacted]

GLP compliance: yes

QA- Reports Yes (X)

Lot and batch numbers: 901

Protocol reviewed by Division No (X):

APPEARS THIS WAY ON ORIGINAL

Methods:

- Species/strain: rat, Crl:CD (SD) BR
- Doses employed: 0, 400, 1200, or 3600 mg/kg/day
- Route of Administration: p.o. by gavage
- Study Design: Dams were dosed from Days 6 to 15 of gestation. Maternal clinical signs, behavior, mortality, food consumption, and body weight were monitored. On Day

20 of gestation, dams were sacrificed and examined for numbers of corpora lutea, implantations, live/dead fetuses, and early/late resorptions. Liver and kidney were also weighed. Fetuses were sexed, weighed, and examined for external anomalies and/or malformations; approximately 50% were examined for skeletal changes and the remaining half for visceral deviations (i.e. Wilson's technique).

- Number of animals/sex/dosing group: 35 pregnant females/group, resulting in 25 to 33 pregnant females evaluated in each group at Day 20 of gestation.

Results:

- Clinical signs: post-dose salivation/wet coats were observed mainly at the high dose level but in all treated groups. Brown staining of the fur was observed in at 1200 and 3600 mg/kg/day in 4/35 and 25/35 animals respectively.
- Mortality: no mortalities.
- Maternal Effects: There was no treatment-related effect on maternal food consumption, body weight, macroscopic findings/organ weights at necropsy. There were no significant differences in pre-treatment events (number of corpora lutea or implantation rate).
- Fetal Effects: Litter size, sex ratio, post-implantation loss, and litter and mean fetal weights were not affected by treatment. Treatment at 3600 mg/kg/day was associated with slight skeletal developmental changes i.e unossified sternebrae and 14th ribs. Treatment at 400 mg/kg/day but not at 1200 mg/kg/day was also associated with higher incidence of fetuses with 14th ribs. In the high dose group, all examined fetuses from 3 litters had a 14 th pair of ribs.

Non-GLP study: An oral teratological study in rats, LE86L251, non-GLP, was conducted at UCB in 1985 in Sprague Dawley rats. 25 females/group at 0, 400, 1200, or 3600 mg/kg/day p.o. by gavage from Days 6 to 15 of gestation, resulting in 21 to 25 pregnant females evaluated in each group at Day 20 of gestation. Same methods as the GLP study. Results different i.e clinical signs in the dams were "nervous behavior" (in most rats) and dirty appearance in several high-dose rats. There were no differences between the groups in the incidence of malformations (visceral and skeletal anomalies) and skeletal variants.

Conclusions: 1200 mg/kg/day was the NOEL for embryofetal development (skeletal variants) and the NOEL for maternal toxicity was 400 mg/kg/day, NOAEL for maternal toxicity was the highest dose i.e. no significant maternal toxicity. There were no skeletal variants in an earlier non-GLP study at a different laboratory.

Study title: A study of the effect of ucb L059 on pregnancy of the rabbit
Study No and Date: RRLE91G0902/UCB 382, January 1992
Site and testing facility: [redacted]
GLP compliance: yes
QA- Reports Yes (X)
Lot and batch numbers: 901
Protocol reviewed by Division No (X)

APPEARS THIS WAY
ON ORIGINAL

Methods:

- Species/strain: New Zealand White rabbits from [redacted]
- Doses employed: 0 or 1800 mg/kg/day

- Route of Administration: p.o. by gavage
- Study Design: Dosed days 6 to 18 of gestation.
- Number of animals/sex/dosing group: time-mated females20/group
- Parameters and endpoints evaluated: Maternal animals were monitored for mortality, clinical signs, food consumption, and body weight. On Day 29 of gestation, dams were sacrificed and examined for abnormalities and macroscopic pathology in maternal organs. The ovaries and uteri were examined for number of corpora lutea and number and distribution of implantations (live young and embryonic/fetal deaths); pre- and post-implantation losses were calculated. Individual fetal/litter weight, external abnormalities, sex, internal abnormalities in the thoracic and abdominal cavities and skeletal deviations were determined in all fetuses.

Results:

- Maternal toxicity: one death, neuromuscular signs (awkward movement of hind quarters, splaying of the hind-limbs, and abnormal posture), piloerection post-dosing, fur loss, soft feces, reduction in food intake during treatment and initial body weight loss, resulting in negative body weight gain.
- Embryofetal toxicity: Decreased fetal weight. Increased incidence of fetuses with 13 ribs, unossified sternebrae, and skeletal anomalies (connected jugal to maxilla, also irregular ossification of cervical vertebral elements).

Non-GLP study: A non-GLP study was conducted at UCB (Report on oral teratological study in rabbits –Segment II, LE85K301, October 1985). Pregnant New Zealand White rabbits (14 to 15/dose) were given levetiracetam at doses 0, 200, 600, or 1800 mg/kg/day p.o. by gavage from Days 6 to 18 of gestation. A positive control group received thalidomide 150 mg/kg/day in similar conditions. The methodology was the same as the GLP study, however some results were different. There was no mortality in the females (one death in the GLP study). Nervousness was noted after dosing in 600 and 1800 mg/kg/day treated females - no neuromuscular signs were noted as was observed in the GLP study. Body weight loss occurred in the 1800 mg/kg/day group during the first 4 days of treatment, resulting in decreased body weight gain (similar to the GLP study). There was a tendency toward lower individual fetal and placental weights at the high-dose level (GLP study had lower fetal weights at the high dose). The number of fetuses with skeletal anomalies (reduced or absent ossification of the 3rd phalanx) was significantly increased at 1800 mg/kg/day. The proportion of fetuses with 13 ribs was higher (though not statistically significantly) in all treated groups. Thalidomide demonstrated the expected embryotoxic and teratogenic effects. The NOEL for maternal toxicity was 600 mg/kg/day and the NOEL for embryofetal toxicity was 600 mg/kg/day.

Conclusion: maternal and embryofetal toxicity at 1800 mg/kg. Similar results between GLP and non-GLP study: maternal clinical signs and weight loss, increased incidence of fetuses with 13th ribs. Different results in this GLP study and a previous non-GLP study: one maternal death in GLP study, reduced/absent ossification of P3 in fetuses at 1800 mg/kg in the non-GLP study.

Study title: A preliminary study of the effect of ucb L059 on pregnancy of the rabbit
Study No and date: RRLE92L2301, December 1992
Site and testing facility[redacted]
GLP compliance: No
Lot and batch numbers: 901
Methods:

- Species/strain: NZW rabbit
- Doses employed: 0, 600, 800, or 1200 mg/kg/day
- Route of Administration: p.o. by gavage
- Study Design: Same as the previous study
- Number of animals/sex/dosing group: 6/group
- Parameters and endpoints evaluated: Same as the previous study except fetal observations were restricted to weight, sex, and macroscopic examination.

Results: Similar to previous study however there were no treatment-related deaths. Neuromuscular clinical signs were again observed (awkward movement of hindquarters with splaying of hind limbs) in mid (800 mg/kg/day) and high dose (1200 mg/kg/day) dams. Lower body weight gain was observed in all treated animals during treatment as in the previous study. There were no treatment-related maternal macroscopic observations, effects on litter parameters or fetuses.

Study Title: A study of the effect of ucb L059 on pregnancy in the rabbit
Study No and date: RRLE91G0902/UCB 386; January 1992
Site and testing facility[redacted]
GLP compliance: yes
QA- Reports Yes (X)
Lot and batch numbers: 901
Protocol reviewed by Division Yes ( ) No (X):
Methods:

- Species/strain: NZW rabbit
- Doses employed: 0, 200, 600, and 1800 mg/kg/day
- Route of Administration: oral by gavage
- Study Design: same as the above studies in rabbits
- Number of animals/sex/dosing group: 16
- Parameters and endpoints evaluated: same as the above studies in rabbits

Results:

- Maternal effects: 4 high dose animals sacrificed for poor body condition following marked signs of inappetance and severe body weight loss also 2 animals aborted. Clinical signs consisting of neuromuscular signs, increased incidence of cold ears, marked reduction in food intake, and reduced body weight gain (including body weight loss up to Day 10) were also observed in the high dose group. The mid-dose group, 600 mg/kg/day, was associated with similar, less marked neuromuscular and food consumption/body weight effects. The low dose group also had neuromuscular signs, decreased food consumption, and decreased body weight gain until Day 14.
- Embryofetal effects: Embryofetal effects as seen in a previous study at this lab were confirmed at the 1800 mg/kg/day dose level. Litter weight and mean fetal weight were

decreased at 1800 mg/kg/day. The incidence of skeletal anomalies was increased at 600 and 1800 mg/kg/day. The incidence of skeletal variations (13th rib) was increased at 1800 mg/kg/day.

Conclusion: Results similar to the above similar studies in pregnant rabbits. The NOEL for maternal toxicity was not determined (below 200 mg/kg/day) and the NOEL for embryofetal toxicity was 200 mg/kg/day.

Study Title: ucb L059 and sodium valproate: an interaction teratology study in the mouse
Study No and date: RRLE98F1501, September 1998
Site and testing facility: [redacted]
GLP compliance: Yes
QA- Reports Yes (X):
Lot and batch numbers: 136
Protocol reviewed by Division: No (X)
Methods:

- Species/strain: Mouse, Ico: OF1 (IOPS Caw)
- Doses/route: 0 or 3000 mg/kg/day levetiracetam p.o. by gavage from gestation Day 6 through Day 15; saline or 400 mg/kg sodium valproate b.i.d. (7 hours apart) were also administered subcutaneously on Day 8 of gestation.
- Study Design: Monitored daily for mortality and clinical signs. Body weights and food consumption were recorded throughout gestation. On gestation Day 18, all dams were sacrificed, uterine contents examined and gross abnormalities noted. The uterus was examined for the number of pregnancies, corpora lutea and implantation sites; number and distribution of live fetuses; number, distribution and classification of embryonic fetal deaths. The fetuses were sexed, weighed and examined for external abnormalities. Pre- and post-implantation losses were calculated.
- Number of animals/sex/dosing group: 25 time-mated females/group. Additional satellite groups (6 time-mated females/treated group) were used for toxicokinetic assessments (Day 8 of gestation).

Results:

- Clinical signs: In the mice administered levetiracetam alone, there were no clinical signs. Mice administered sodium valproate (alone or in combination with levetiracetam) developed sores or scabs around the injection site, and subdued behavior often with prostration.
- Mortality: none.
- Body weight: Maternal body weights were unaffected by treatment.
- Food consumption: Mean maternal food consumption was significantly decreased in all drug-treated dams between Days 8 and 11 of gestation. In the animals receiving sodium valproate statistically significant food consumption reduction was also noted between Days 16 and 18 of gestation.
- Fertility and Embryo-fetal effects: There were no treatment-related effects of levetiracetam on pregnancy rates, embryonic and fetal resorptions, pre- and post-implantation losses, fetal sex ratio , fetal weight, fetal malformations and macroscopic examination of the dams. In the groups administered sodium valproate, the

number of late resorptions and post-implantations losses were increased, mean litter sizes and fetal weights were lower, and dead fetuses were noted. Sodium valproate produced the expected fetal malformations.

Preliminary range-finding studies in the mouse: (**ucb L059 and sodium valproate - dose range-finding teratology study in the mouse**, RRLE98A3002, May 1998) GLP study at [redacted] female OF1 mice (8/group) given levetiracetam 0, 1000, and 3000 mg/kg/day p.o. from Days 6 through 15 of gestation. Based on the absence of maternal toxicity or effect on fetal development among these animals, 3000 mg/kg/day was selected for use in a second experiment to investigate the effects of levetiracetam alone or in conjunction with sodium valproate - OF1 mice (8/group) were administered daily doses of levetiracetam 3000 mg/kg/day p.o. alone or in combination with s.c. sodium valproate 100 or 400 mg/kg/day. Levetiracetam was administered on Days 6 through 15 of gestation and sodium valproate was administered once on Day 8 of gestation. Controls were administered water by gavage and s.c. sterile saline. Positive controls were administered only sodium valproate. Same maternal/embryonic parameters examined. Oral administration of levetiracetam at up to 3000 mg/kg/day from Day 6 to 15 of gestation in mice had no obvious maternal toxicity, and no effects on development of embryos. The administered dose of valproate (100, 400 mg/kg/day on Day 8) did not induce the expected incidence of fetal malformation.

Conclusions: Not embryotoxic in the mouse when administered orally at a dose level of 3000 mg/kg/day from Days 6 through 15 of gestation. Levetiracetam did not potentiate the teratogenic effects of sodium valproate.

Study title: A study of the effect of ucb L059 on the pregnant rat and offspring during the peri and post natal period
Study No and date: RRLE91G1001, November 1991
Site and testing facility: [redacted]
GLP compliance: yes
QA- Reports Yes (X)
Lot and batch numbers: 901
Protocol reviewed by Division No (X)
Methods:

- Species/strain: Sprague Dawley rats
- Doses employed: 0, 70, 350, or 1800 mg/kg/day
- Route of Administration: p.o. gavage
- Study Design: Time-mated. Given drug levetiracetam from Day 15 of pregnancy to Day 21 *post-partum*.
- Number of animals/sex/dosing group: 25/group
- Parameters and endpoints evaluated: Females allowed to litter and rear young to weaning then sacrificed and subjected to macroscopic examination. Parent females were monitored for clinical signs, food consumption, body weight, and duration of pregnancy. Offspring were counted, weighed, sexed, and examined for external abnormalities after parturition. During the pre-weaning period, pups were examined for developmental effects *via* surface righting reflex, startle reflex, air righting reflex, and pupil reflex. At

weaning, offspring were sacrificed and examined macroscopically. Liver and kidneys from dams were weighed.

Results:

- Maternal effects: Treatment-related clinical signs (post-dosing salivation/wet coats) were observed at 350 and 1800 mg/kg/day. There were no other treatment-related maternal effects.
- Prenatal and postnatal development, including maternal function: There were no treatment-related effects on litter parameters, pre-weaning development, or *post-mortem* examination.

Preliminary study: **A preliminary study of the effect of ucb L059 on the pregnant rat and offspring during the peri- and post-natal period** (RRLE91B0801, July 1991).
Preliminary non-GLP study conducted at [redacted] same species/strain, dose, methods, and results all the same.

Conclusions: NOEL for maternal toxicity and peri- and post-natal development was the highest dose (no maternal toxicity, no developmental abnormalities).

Study title: Pharmacokinetic aspects in Sprague-Dawley rats
Study No and date: LE86H072
Site and testing facility: UCB, Chemin du Foriest, Belgium
GLP compliance: no
Lot and batch numbers: 12
Protocol reviewed by Division No (X)
Methods:

- Species/strain: Sprague Dawley rats
- Doses employed: 0, 54, 400, 1200, 3600 mg/kg/day
- Route of Administration: p.o. gavage
- Study Design: Plasma kinetics, urinary excretion
- Number of animals/sex/dosing group: 5

Results/conclusions: Males, females similar. Rapid and complete absorption at all doses; rate of absorption reduced as dose increased. 80-90% of the dose excreted in urine. AUCs were directly linearly proportional to the doses. AUCs (sexes combined) for 54, 400, 1200 and 3600 mg/kg (ratios 1, 7.5, 22, and 66) were respectively 286, 1842, 5564, 16502 ug/ml (ratios 1, 6.5, 19, and 58).

Study title: Pharmacokinetic aspects in New Zealand rabbits
Study No and date: LE86M031
Site and testing facility: UCB, Chemin du Foriest, Belgium
GLP compliance: no
Lot and batch numbers: 12
Protocol reviewed by Division No (X)
Methods:

- Species/strain: New Zealand white rabbits
- Doses employed: 0, 200, 600, 1800 mg/kg/day
- Route of Administration: p.o. gavage

- Study Design: Absorption, plasma kinetics, urinary excretion at the three doses above (also absorption, distribution, metabolism, excretion studies of a single dose 54 mg/kg iv and po)
- Number of animals/sex/dosing group: 3/sex/group (also 4 pregnant/4 non-pregnant)

Results/conclusions: ADME similar in pregnant and non-pregnant rabbits. Fetal and placental concentrations are very close to maternal plasma levels, crosses placental barrier, does not accumulate in amniotic fluid. Rapid and complete absorption at all doses; rate of absorption reduced as dose increased. 50-70% of the dose excreted in urine. C max and AUCs were directly linearly proportional to the doses. Males, females similar. AUCs (sexes combined) for 200, 600 and 1800 mg/kg (ratios 1, 3, 9) were respectively 2190, 6280, 19638 ug/ml (ratios 1, 2.8, 9). See Table 6 below. For exposure comparisons, refer also to Table 7 below. The AUC (0-∞) in rabbits at the highest dose exceeds that in the human highest dose by 25x.

Table 6: Plasma Pharmacokinetics of Levetiracetam as a Function of Single Oral Dose in the Rabbit♣

Parameter / Dose	200 mg/kg	600 mg/kg	1800 mg/kg
Half-life (hrs)	3.8	3.7	3.7
T_{max} (hrs)	1.1	1.3	1.6
C_{max} (ug/mL)	295	797	2291
Normalized C_{max}	1.5	1.3	1.3
AUC 0-∞(ug-h/mL)	2190	6280	19638
Normalized AUC*	11.0	10.5	10.9

♣Mean values for males/females combined
*equated for a 1 mg/kg dose

Table 7: Plasma Pharmacokinetics of Levetiracetam as a function of Repeat Oral Dosing in Humans (Study N202, healthy volunteers, day 7 of dosing)

Parameter/Dose	1000 mg b.i.d.	1500 mg b.i.d.
T_{max} (hrs)	2.8	2.5
C_{max} (ug/mL)	36.3	52.0
AUC 0-∞(ug-h/mL)	560.3	785.8

Summary and Evaluation of Reproductive Toxicology: Administration of levetiracetam prior to mating and up to the end of pregnancy or lactation did not effect reproductive performance or fertility. Similarly, administration of levetiracetam to pregnant rats or mice during the period of organogenesis was associated with only minor clinical signs in dams, *e.g.*, post-dose salivation or body weight effects. Embryo-fetal effects observed in teratology studies in rats and mice were primarily limited to decreased fetal weight, growth retardation, and increased incidence of delayed ossification parameters at 3600 mg/kg in rats and 3000 mg/kg in mice and differences in rib number in rats. The delay in ossification was related to slower growth rate as evidenced by decreased fetal weights and slightly lower maternal body weight gain at the end of pregnancy. Effects on fetal growth were not seen at 1800 mg/kg/day where pregnancy was conducted to term and there were no effects on survival, litter parameters, or development of the offspring during the peri- and post-natal period. In one rabbit teratology study, there was a two fold increase in

incidence of spontaneously occurring abnormalities at 1800 mg/kg/day, a dose which was markedly toxic to the dams. This was not seen in the other two studies using this dose. Administration of levetiracetam at 3000 mg/kg/day in pregnant mice did not affect the teratogenic effect of valproate.

GENETIC TOXICOLOGY:

In vitro evaluations included tests for gene mutations in bacteria and eukaryotic systems and chromosomal aberrations in mammalian cells. A micronucleus test was conducted *in vivo* in mice. All of these studies were conducted in compliance with GLP guidelines and included appropriate positive and negative controls. The following studies were previously reviewed and considered to be negative [redacted] Ames metabolic activation test to assess the potential mutagenic effect of ucb L059, An assessment of the mutagenic potential of ucb L059 in mammalian cells *in vitro* using the Chinese hamster ovary/HGPRT locus assay, and Analysis of metaphase chromosomes obtained from CHO cells cultured *in vitro* and treated with ucb L059.

The previous reviewer noted that The S. typhimurium tester strains did not include all the recommended strains, specifically TA 102 (for A-T point mutations). However the recent *Escherichia coli* WP2uvrA study is a satisfactory equivalent according to current ICH guidelines - this recent study, not previously reviewed, is reviewed here (see Testing for mutagenic activity with *Escherichia coli* WP2uvrA).

The previous reviewer (L. Freed) requested documentation of adequate exposure (plasma or bone marrow) in the mouse micronucleus test which was not submitted in this NDA. However based upon known pharmacology and pharmacokinetics in the mouse, and the abundance of dose related toxic clinical signs documented in the dose groups in this micronucleus test, adequate exposures were clearly achieved and further documentation is not necessary.

The previous reviewer (L. Freed) also questioned plate contamination in the CHO/HGPRT. The sponsor included a report amendment (dated 29 April 1996) in this NDA which satisfactorily explains this. The contamination was due to fungal contamination which occurred randomly and is a commonly occurring event in such studies – those plates were unreadable and discarded. This was a valid and acceptable study.

A number of genetic toxicology studies were also conducted on two metabolites, ucb L057 (the major human and animal metabolite) and ucb L060 (a minor metabolite in humans and animals, present as an impurity in the manufacturing process. All assays were negative (see attached review by Aisar Atrakchi, 10-22-99).

Study Title: Testing for mutagenic activity with *Escherichia coli* WP2uvrA
Study No: RRLE96L0604, report date March 1997
Volume # and Page #: 65, 17781
Conducting Laboratory: [redacted]
Date of Study Initiation/completion: 23-25 Sep 1996
GLP Compliance: yes

Drug Lot Number: 136
Methodology:

- Strains/Species/Cell line: *E. coli* WP2uvrA, male F344 rats
- Metabolic Activation System: Aroclor 1254 induced S9 enzymes
- Controls:
  - Vehicle: sterile water
  - Negative Controls: none
  - Positive Controls: ENNG and 2-ANN in DMSO
- Exposure Conditions/Incubation and sampling times: Bacteria were preincubated with test material for 20 minutes in the presence or absence of an Aroclor 1254-induced rat liver S9 mix before mixing with top agar and plating. Plates were incubated for 48 hours in the presence/absence of S9 mix to assess the effect of metabolic activation.
- Doses used in definitive study: Levetiracetam was tested as a solution in water at concentrations of 0, 156.25, 312.5, 625, 1250, 2500, and 5000 ug/plate.
  - Study design: Two independent mutation tests were performed.
- Criteria for Positive Results: At least a doubling of vehicle control values, a dose-response (except at very high doses), reproducible in independent tests.

Results:

- Study Validity: acceptable
- Study Outcome: Levetiracetam was negative in this assay. The positive control caused the expected increase in colony numbers.

Summary of Genetic Toxicology: Standard battery of genetic toxicology tests adequate, acceptable and negative.

SPECIAL TOXICOLOGY STUDIES:

General Comments: Special Studies with Levetiracetam were previously reviewed; [redacted] Ucb L059-induced male rat-specific tubular nephropathy role of alpha-2-microglobulin; Ucb L059 investigation of the role of alpha-2-microglobulin in renal changes following oral administration to Sprague-Dawley rats; Cytotoxicity of ucb L059, carbamazepine, phenytoin, and valproic acid on primary cultures of rat hepatocytes.

OVERALL SUMMARY AND EVALUATION:

Introduction:
Levetiracetam is a **new chemical entity** and the active ingredient of Kepra tablets. Levetiracetam is the S-enantiomer of a-ethyl-2-oxo-1-pyrrolidine acetamide and is also referred to as ucb L059 or ucb 22059. It is chemically related to piracetam, a compound used in Europe for more than 25 years for the treatment of various cognitive disorders. The intended use is in adult epileptic patients, as adjunctive therapy in refractory partial onset seizures with and without secondary generalization. The recommended dose range is 1000 to 3000 mg/day in a b.i.d regimen i.e. 20 to 60 mg/kg/day for a 50 kg patient. All excipients have been used previously in oral formulations.